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# The acid-base condition in vegetation, litter and humus: V. Products of partial oxidation and ammonia fixation.

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Litter and humus undergo a rapid auto-oxidation when exposed to the air in an alkaline medium. The content of acids and acidoids and the power to fix ammonia are thereby greatly increased (cf. Part III and IV of this series). In view of this fact it was decided to make a detailed study of the progressive, partial oxidation of organic matter by mild chemical oxidants as well as by auto-oxidation, to study its effect on the fixation of ammonia and to examine the products obtained. The following is a report of the results thus far obtained.

In the following work all titrations have been made in N. 1 KCl solution instead of water as in our previous work. This increases the capacity to bind base at any given pH and yields, therefore, a higher acidoid content.

## Oxidation with hydrogen peroxide.

The following experiments on oxidation with hydrogen peroxide have been performed:

Materials:

Häggbygget $F_2$ humus, 20 grams (dry basis).
Hydrogen peroxide 30 % solution.
Solution volume 400 cc.

Series A: Heated 18 hours in a steel bomb at 132° C.

1. 0 cc $H_2O_2$ + 400 m.e. NaOH.
2. 10 » » + » » »
3. 20 » » + » » »

Series B: At room temperature 8 days in vacuum flasks of 2 li-

ters capacity. Peroxide introduced twice daily in 5 cc portions through a special tube provided with double stopcocks.

1. 10 cc $H_2O_2$.
2. 10 » » + 25 m.e. NaOH.
3. 10 » » + 100 » »
4. 10 » » + 400 » »
5. 10 » » +1600 » »

Series C: Treatment same as B except system 1 which was kept (anaerobic) in a 400 cc stoppered flask and system 7 which was evacuated and filled with oxygen gas (= about 1900 cc).

1. 0 cc $H_2O_2$ +400 m.e. NaOH.
2. 5 » » + » » »
3. 10 » » + » » »
4. 20 » » + » » »
5. 30 » » + » » »
6. 40 » » + » » »
7. $O_2$ gas + » » »

Series D: Treatment same as B.

1. 5 cc $H_2O_2$.
2. 10 » »
3. 20 » »
4. 40 » »

All flasks were occasionally shaken.

Systems C 6 and 7 were each connected to a manometer. Observations: In system C 6 the pressure decreased by as much as 10 mm Hg until 15 cc of the peroxide were added after which the pressure rose increasingly after each addition, registering 550 mm on the eighth day. The pressure of the oxygen gas in system C 7 fell from normal to 380 mm on the eighth day.

At the end of the treatment the containers were connected to a vacuum flask containing standard alkali and the free carbon dioxide was drawn through a wash tower and then into the alkali solution through a sintered glass immersion filter. The systems containing alkali received, after a preliminary evacuation, a calculated excess (to n. 0.05) of $H_2SO_4$, which can be safely introduced by suction. The last trace of carbon dioxide was then expelled by placing the flask containing the humus in a waterbath with a maximum temperature of about 50° C regulated so that the boiling is not too violent. To avoid any possible loss of unadsorbed carbon dioxide it is best to close the connection between the flasks each time the flask containing the alkali is evacuated. When all the air has been expelled no further evacuation is necessary, the closed system work-

ing smoothly because of the difference in temperature. The end point is quite evident.

An aliquot of the alkali solution was then titrated to phenolphthalein in the presence of an excess of barium chloride and the $CO_2$ calculated in terms of millimols per 100 grams original organic matter after correction for the blank analysis.

The contents of the flasks were then filtered through a hard filter paper and washed with water until a filtrate of 2 liters was obtained. The residues were then electrodialysed and dried. The $pH_u$ and the titration in N.KCl with KOH were determined. The capacity to bind base at pH 7 is put equal to the acidoid content, $A$, and expressed as m.e./100 g organic matter.

The clear filtrates, the color of which was mostly quite dark, contain the simple organic acids ($a$-acids) and, of course, a multitude of nonacidic, water soluble organic compounds. But it contains also a more or less distinct groups of highly dispersed humic acids which are not precipitable by mineral acids but which may be precipitated by Al and Fe and other heavy metal cations. These possess, as we shall see, an enormous capacity to bind base and represent, probably, largely that humic acid fraction in the soil, notably in the podzol, which is leached out of the A-horizon and is precipitated with the sesquioxides in the B-horizon. In passing a solution of these acids through a soil material rich in sesquioxides they may, at the proper reaction (slightly acid) be completely adsorbed (isoelectrically precipitated).

Because of this relationship to the development of the B-horizon they might be given the name $\beta$-humic acids to distinguish them from the truly colloidal humic acids (acidoids) which are precipitable by acids and which, for this reason, largely remain in the A-horizon and might be called $\alpha$-humic acids. This recalls Waksmans $\alpha$- and $\beta$-humus (1938) but the distinction must not be overlooked that Waksman applies the name $\beta$-humus to humic acids which were soluble in acids when *in combination* with aluminum. All humic acids, $\alpha$ as well as $\beta$, form amphoteric humates which are soluble (i.e., dispersible) on the acid side of their I. E. P. We would only group as $\beta$-acids such humic acids which in the *free* state are acid-soluble, otherwise the definition has no genetic significance. In the following we shall continue to denote the $\alpha$-acids (acidoids) by the letter $A$, the simple, nonprecipitable organic acids by the letter $a$ and the acid-soluble but sesquioxide-precipitable acids by the letter $\beta$.

The estimation and separation of the $\beta$-acids from the filtrate was carried out as follows:

An aliquot of the filtrate was diluted and neutralized to phenolphthalein by NaOH. It was then evaporated to dryness, weighed and ignited and the »excess base» in the residue was determined by

backward titration of the acidified solution. The value thus obtained is put equal to $a+\beta$, i. e., the sum of the simple organic acids and the $\beta$-acids. To another aliquot of 100 cc, 10 or 20 m. mols aluminum sulfate were added and the reaction adjusted to the highest pH which would cause a complete precipitation. (At a pH somewhat above the I. E. P. and in the presence of sulfate ions the simple organic anions will not be carried down as saloid-bound ions to any appreciable extent but will be displaced by the $SO_4$ and OH ions.) This leaves a colorless, clear solution. From the known total volume a measured aliquot of this solution was withdrawn, neutralized to phenolphthalein, evaporated to dryness, weighed, ignited and the »excess base» determined as before. The value thus obtained is put equal to the simple organic acids not precipitated by Al. The content of $\beta$-acids was then obtained by the difference $(a+\beta)-a=\beta$. These values are all expressed in table 29 in terms of m.e./100 g of original organic matter.

The loss on ignition of the residues obtained by evaporating the original and the fractionated filtrates, from which the weights of the $a$ and $\beta$-acids (strictly $a$ and $\beta$ organic matter) were obtained, was corrected by adding 30 mg (= 1 m.e. $CO_3$) for each m.e. excess base in the ignited residue. These corrected values are given in terms of grams per 100 grams of original organic matter.

The $a$ and $\beta$ fractions were separated only in the A and C series. In the systems which did not contain an excess of alkali it was found that the peroxide continued to decompose the organic matter in the filtrates after the carbon dioxide determination. When the neutralized filtrates were evaporated there was an evident action of the still undecomposed peroxide. This caused a loss of organic matter and made the sum: grams $a+\beta$ too low. These values are given, for what they may be worth, in table 29 but have been omitted in the calculations in table 30.

The most notable results of the partial oxidation by the peroxide may be summed up as follows:

*$a$ and $\beta$-acids*: Large amounts of these acids are formed, the quantities increasing with the increase of peroxide and with the alkalinity of the medium (table 29). In the bomb at 132° C. (A series) the $\beta$-acids (m.e.) exceed the $a$-acids (in the anaerobic A 1 by as much as 100 %) whereas at normal temperature and pressure the $a$-acids are in excess (C series). The milder oxygen gas yields much more $\beta$- than $a$-acids (C 7). In the absence of base (series D and system B 1) and in the presence of insufficient base to render the system alkaline (B 2) there is less acids but relatively more carbon dioxide is formed. This significant fact will be discussed below.

The capacity per 100 grams of the acids, i.e. organic matter, to bind base (table 30) is surprisingly high. Thus system C 6 with

Table 29. *The products of a partial oxidation of humus (Häggbygget podzol $F_2$) by hydrogen peroxide. $a$ = soluble organic acids not precipitable by Al ions; $\beta$ = soluble (colored) organic acid precipitable by Al ions; $A$ = acidoids in the insoluble residue.*

| System | Treatment | | Per 100 g. original organic matter: | | | | | | | Increase $a+\beta+A$ (y)[1] | $\frac{CO_2}{a+\beta+A} = \frac{x-3}{y}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30 % $H_2O_2$ | NaOH | Soluble organic matter | | Insol. residue | $CO_2$ (x) | $a$ | $\beta$ | $A$ | | |
| | | | $a$ | $\beta$ | | | | | | | |
| | cc | m.e. | g. | g. | g. | m.mols | m.e. | m.e. | m.e. | m.e. | |
| O | 0 | 0 | 2.9 | 0.5 | 88 | 3 | 3 | 3 | 115 | — | — |
| A 1 | 0 | 400 | 18.6 | 21.4 | 48 | 77 | 60 | 119 | 83 | 141 | .52 |
| A 2 | 10 | 400 | 24.7 | 26.0 | 43 | 167 | 203 | 239 | 81 | 402 | .41 |
| A 3 | 20 | 400 | 29.6 | 26.7 | 36 | 262 | 276 | 293 | 68 | 516 | .50 |
| B 1 | 10 | — | 11.8 | | 75 | 65 | 76 | | 116 | 71 | .87 |
| B 2 | 10 | 25 | 11.4 | | 70 | 64 | 70 | | 107 | 56 | 1.09 |
| B 3 | 10 | 100 | 22.7 | | 64 | 89 | 163 | | 118 | 160 | .54 |
| B 4 | 10 | 400 | 32.9 | | 54 | 95 | 243 | | 109 | 231 | .40 |
| B 5 | 10 | 1600 | 48.0 | | 49 | 103 | 349 | | 87 | 315 | .32 |
| C 1 | 0 | 400 | 10.7 | 6.4 | 69 | 15 | 32 | 42 | 113 | 66 | .18 |
| C 2 | 5 | 400 | 14.6 | 9.2 | 59 | 62 | 87 | 74 | 115 | 155 | .38 |
| C 3 | 10 | 400 | 19.3 | 13.5 | 54 | 95 | 135 | 108 | 109 | 231 | .40 |
| C 4 | 20 | 400 | 30.9 | 18.3 | 40 | 171 | 283 | 164 | 69 | 395 | .43 |
| C 5 | 30 | 400 | 33.8 | 23.4 | 38 | 215 | 322 | 261 | 61 | 523 | .41 |
| C 6 | 40 | 400 | 33.8 | 28.9 | 33 | 259 | 351 | 332 | 50 | 612 | .42 |
| C 7 | $0_2$ | 400 | 13.9 | 28.4 | 53 | 99 | 73 | 213 | 100 | 265 | .36 |
| D 1 | 5 | — | 6.1 | | 82 | 19 | 23 | | 113 | 15 | 1.07 |
| D 2 | 10 | — | 11.8 | | 75 | 65 | 76 | | 116 | 71 | .87 |
| D 3 | 20 | — | 18.0 | | 68 | 96 | 162 | | 104 | 145 | .64 |
| D 4 | 40 | — | 20.7 | | 62 | 134 | 189 | | 97 | 165 | .79 |

[1] Obtained by subtracting $a+\beta+A$ in system O.

1149 m.e. per 100 grams of the $\beta$-acids yields an equivalent weight of only 87. This low value for the equivalent weight is probably due to the formation of polycarboxylic acids of benzene such as mellitic acid (benzene hexacarboxylic acid, $C_6(CO_2H)_6$). This acid occurs in peat in the form of its aluminum salt (»honey-stone», $C_{12}Al_2O_{12}\cdot 18H_2O$). It is also formed by the oxidation of lignite or graphite with $KMnO_4$. The senior author has collected considerable quantities of this acid from the anode compartment in his electrodialysis cell when, several years ago, graphite anodes were used. The dialysate, black with colloidal carbon, was precipitated with calcium hydroxide and was found to possess an enormous »base exchange» capacity. Later, fine silky needles of mellitic acid were obtained from the black precipitate.

Table 30. *The base binding capacities of the acids and acidoids (per 100 g organic matter) and the pHu and nitrogen content of the electrodialysed residue.*

| System | Per 100 g. organic matter: | | | | | | $pH_u$ in N. KCl |
|---|---|---|---|---|---|---|---|
| | $\alpha$ m.e. | $\beta$ m.e. | $A$(pH 7) m.e. | $A$(pH 10) m.e. | $\frac{A(\text{pH 10})}{A(\text{pH 7})}$ | N % | |
| O | (103)[1] | (600)[1] | 131 | 290 | 2.21 | 1.77 | 2.18 |
| A 1 | 323 | 556 | 173 | 364 | 2.10 | .83 | 3.32 |
| A 2 | 822 | 919 | 189 | 356 | 1.88 | .84 | 2.26 |
| A 3 | 932 | 1097 | 189 | 322 | 1.70 | .80 | 2.20 |
| B 1 | — | — | 155 | 350 | 2.26 | 1.81 | 2.06 |
| B 2 | — | — | 153 | 332 | 2.17 | 1.80 | 2.00 |
| B 3 | — | — | 183 | 296 | 1.62 | 1.94 | 1.85 |
| B 4 | — | — | 201 | 308 | 1.53 | 1.70 | 1.84 |
| B 5 | — | — | 179 | 292 | 1.63 | 1.26 | 1.92 |
| C 1 | 299 | 656 | 164 | 339 | 2.07 | 1.57 | 2.01 |
| C 2 | 596 | 804 | 193 | 328 | 1.70 | 1.67 | 1.95 |
| C 3 | 699 | 800 | 201 | 308 | 1.53 | 1.70 | 1.84 |
| C 4 | 916 | 896 | 174 | 252 | 1.45 | 1.80 | 1.95 |
| C 5 | 953 | 1115 | 161 | 233 | 1.45 | 1.77 | 2.01 |
| C 6 | 1038 | 1149 | 153 | 215 | 1.41 | 1.82 | 2.07 |
| C 7 | 525 | 750 | 190 | 291 | 1.53 | 1.64 | 1.82 |
| D 1 | — | — | 138 | 331 | 2.40 | 1.76 | 2.07 |
| D 2 | — | — | 155 | 350 | 2.26 | 1.81 | 2.06 |
| D 3 | — | — | 154 | 342 | 2.22 | 1.81 | 2.01 |
| D 4 | — | — | 157 | 340 | 2.17 | 1.87 | 2.04 |

[1] Uncertain values due to small amounts.

This does not mean that we identify the $\beta$-acids with mellitic acid. The chemistry of humus is not so simple. But there is no doubt that polycarboxylic acids of benzene are formed as intermediate products when a complex containing condensed benzene rings is oxidized.

The fact that the combining capacity of the $\alpha$-acids is, in general, lower than that of the $\beta$-acids must be ascribed to the presence of nonacidic soluble organic matter with which the $\alpha$-acids are »diluted».

*Acidoids*: The acidoid content ($A$ in table 29) in the O-system is 115 m.e./100 g. This value has maintained itself in several of the systems despite the loss in the weight of the insoluble residue. These systems include those to which none, or but little alkali was added and which received only 5 or 10 cc peroxide. The original acidoid content has also maintained itself in systems C 1 and C 2 which received excess of alkali but 0 and 5 cc peroxide respectively. In the bomb at 132° C the acidoid content decreased, even in A 1 which received no peroxide. The acidoid content of the systems decreases with an increase in $\alpha$- and $\beta$-acids.

The acidoid content per 100 grams organic matter in the residues (column 4, table 30) has increased in every system as compared to that of the original material which is 131 m.e. (system O). The increase in acidoids without peroxide (systems A 1 and C 1) must be ascribed to an intramolecular oxidation-reduction or to a hydrolysis or both. Without alkali the acidoids nowhere exceed 157 m.e. (D 4, an increase of 26 m.e.). In an alkaline medium the acidoid content reaches a value of 201 m.e. (B 4 = C 3, an increase of 70 m.e.).

The maxima in acidoid content at moderate concentrations of alkali or peroxide in systems B and C demand some comments. In the presence of much alkali (B 5) and in the presence of moderate alkali but much peroxide (C 4 to C 6) the residues appeared bleached and spongy. It was obvious that the acidoids in these residues were relatively much more »diluted» by an inert skeleton of nonacidic material.[1] The acidoid groups had apparently more extensively been split off in the form af $\alpha$ and $\beta$-acids than in the case of the less drastically treated systems.

With due allowance for this fact we find that chemical oxidation leads to a high acidoid formation only in an alkaline medium exactly as under natural conditions we find a high acidoid content associated with a high base status (cf. Part III).

The capacity to bind base at pH 10 shows a different trend. It is highest (364 m.e.) in the heated system without peroxide (A 1) and in the C series it is highest in C 1. This points to an increase in the number of free phenol groups by the action of the alkali, due possibly to a displacement of some of the methoxyl groups (cf. below). It is interesting to note that peroxide in the presence alkali causes a decrease in the capacity to bind base at high pH. Thus the capacity at pH 10 decreases with an increase in peroxide in every series except the acidic D series. The ratios $A$(pH 10): $A$(pH 7) decrease accordingly with an increase in peroxide. It appears as if oxidation causes the condensed benzene rings to break at the phenol group. The so produced side chains are then easely oxidized with the result that the ring aggregate is broken up into a number of fragments giving rise to simpler acidoids of high capacity, to $\beta$-acids, to simple organic acids and to carbon dioxide. Further evidence of this mode of decomposition will be presented later.

The titration curves are shown in fig. 30. The curve of system O is plotted in each series for comparison.

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[1] If the acidoids were extracted by alkali from the inert skeleton they would undoubtedly show a much higher capacity to bind base, and one which would probably increase in the same order in which the capacities of the $\beta$-acids increase. We have isolated the »pure» acidoids from different forms of humus and from different treatments and are now studying the composition and behavior of these products.

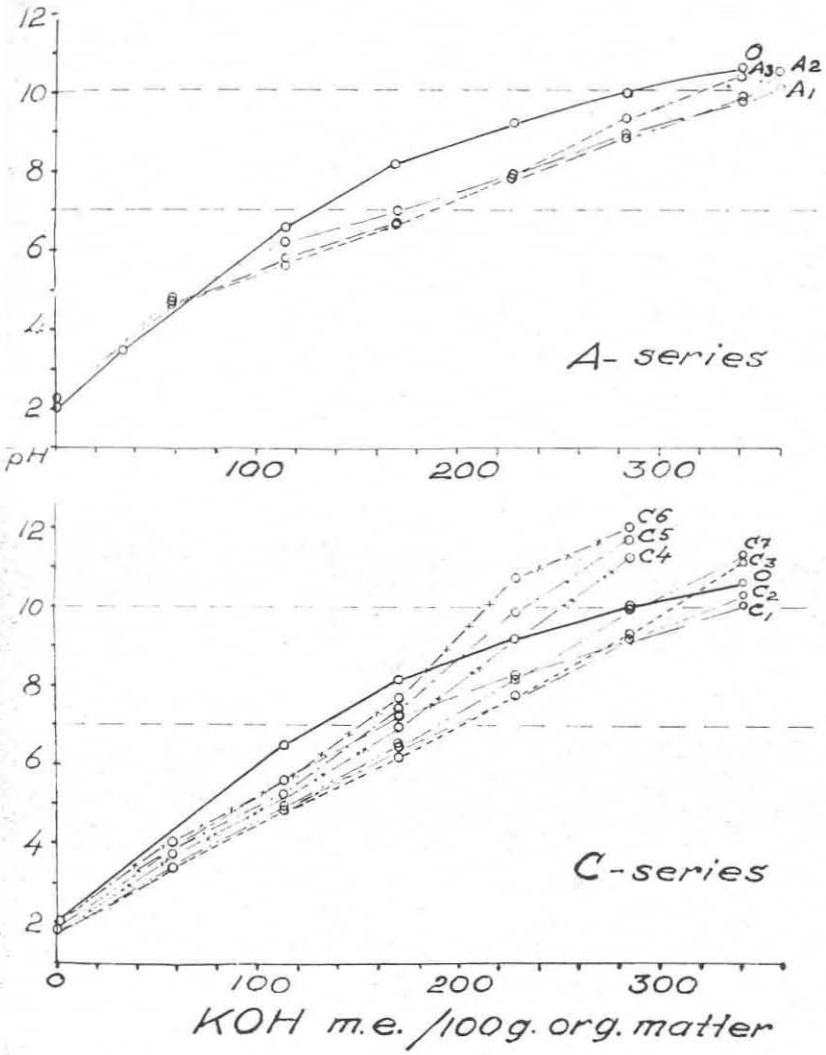



Fig. 30. The titration curves of the electrodialysed samples in series A and C and in system O in table 30.

*The increase in acidity*: This is obtained by subtracting the sum of the m.e. $a+\beta+A$ in the O system (= 121) from the corresponding sums of the treated systems. The increase varies between 15 (D 1) and 612 (C 6) m.e. (table 29).

$CO_2$: The formation of carbon dioxide varies between 15 (C 1) and 262 (A 3) millimols in the treated samples (table 29).

*$CO_2/(a+\beta+A)$ ratio*: The fact that a high base status is associated with a high acidoid content, and vice versa, led us to the conclusion that »the quantity of acidoids must be governed by an equilibrium between two steps in the oxidation of organic matter. In the first of these the acidoids are formed and in the second they are decomposed (to $CO_2$ and $H_2O$). The relative rate of these two oxidations must fix the acidoid level of the system, and any factor, e.g., the base status, which affects the two steps of oxidation differently will cause a change in the acidoid level» (cf. Part III of this series). The ratios between the $CO_2$ and the sum $a+\beta+A$ obtained by the various treatments support this conclusion (table 29). Thus in the acidic systems (B 1, B 2 and D series) the proportion of $CO_2$ to acids is about twice as great as in the alkaline systems. This indicates that the carboxyl groups are more easely oxidized to $CO_2$ in an acid than in an alkaline medium. In »sour» humus the acidoid content can, therefore, never attain the same level we find in the »mild» humus.

*$pH_u$ in N. KCl solution.* The pH values of the electrodialysed residues in N. KCl are all, with the exception of the A series, lower than those of the untreated sample. The heating in the bomb seems to weaken the acidoids, despite the fact that the nitrogen content in the A series has been greatly reduced. With the exception of the A series the $pH_u$ values all fit the curve in fig. 40 surprisingly close (cf. discussion below).

*The N-content*: This is, as already pointed out, greatly reduced when the humus is heated in the presence of alkali. It is also reduced by strong alkali without heating (B 5). In the oxidized systems to which either no alkali or only small amounts were added the nitrogen content has maintained itself or has been somewhat increased. There is evidence that oxidation counteracts the deaminating effect of the alkali (cf. series C, cf. also $NH_3$-fixation below).

*The color of the β-acids*: It is our intention to make a special study of the color and the light adsorption of the various humates we have been preparing. We shall here merely mention some observed color reactions of the β-acids.

Table 31 shows the relative intensity of coloration of the filtrates of the A and C series before and after the addition of an excess of alkali, and the »coefficient of darkening» produced by the alkali. The coloration of the alkaline C 1 is used as standard (= 1).

The most noteworthy thing in this table is the fact that the more thoroughly oxidized the system the smaller is the coefficient of darkening when the solution is alkalinized. The anaerobically heated system A 1 became 8.5 times darker when NaOH was added in excess. (In ammonia it became 10.5 times darker.)

It was then observed that the alkalinized A 1 and, to a less extent, the A 2 and A 3 solutions rapidly paled when exposed to the

Table 31.

| System | Coloration of filtrates: | | Coefficient of darkening |
|---|---|---|---|
| | acid | alkaline | |
| C 1 | .25 | 1.00 | 4.0 |
| C 2 | .38 | 1.25 | 3.3 |
| C 3 | .81 | 2.27 | 2.8 |
| C 4 | 1.07 | 2.78 | 2.6 |
| C 5 | 1.02 | 2.50 | 2.5 |
| C 6 | .92 | 2.12 | 2.3 |
| C 7 | 2.27 | 5.0 | 2.2 |
| A 1 | .78 | 6.67 | 8.5 |
| A 2 | 1.15 | 5.55 | 4.8 |
| A 3 | 1.20 | 4.0 | 3.3 |

air. The color changes at the same time from wine brown to a yellowish brown. The paling of the A 1 solution was observed by placing 100 cc in a stoppered 5 liter Erlenmeyer. It was very rapid in the first hour during which the coefficient of darkening had fallen from 8.5 to 6. After 114 hours it was 2.58 and after 162 hours 2.56 or about the same as the well oxidized C series. Upon reacidification the solution is somewhat darker than the original acid solution and yields a precipitate. The color of humus and its changes as a result of various treatments might be useful in the study of humus but it is obviously of little value in the quantitative estimation of humus.

It deserves to be mentioned that the alkalizined filtrate from A 1 adsorbed, contrary to our expectations, less than half as much oxygen as the filtrates from the oxidized samples, indicating that the anaerobic heating desactivates the reducing power, possibly through a polymerization.

The titration curves of the filtrates show great buffer capacity at low pH but begin to get quite steep already at pH 6. This shows that the fragments which are split off from the mother complex contain the strongest acid groups.

## Oxydation by oxygen.

### a. Original samples.

This oxydation was carried out by placing the material, usually 20 grams, in a 2 liter vacuum flask together with 400 cc of solution, evacuating the flask and filling it with oxygen. During the absorption of the gas the flasks were shaken in the machine and the amount consumed was measured at intervals by connecting the flask to a 500 cc gas burette containing oxygen.

The following preliminary experiments are of interest.

Twenty grams of humus (beech leaves, F layer) in 400 cc N. 1 NaOH were oxidized as follows:

a. The flask was evacuated and the oxygen was added immediately after the addition of the alkaline solution.

b. The humus was kept in the alkaline solution for 24 hours before the oxygen was added.

c. The humus was alkalinized six days before the oxygen was added.

d. The evacuated flask containing the humus and the alkaline solution was placed in a water bath and boiled for four hours and then left to cool over night before the oxygen was added.

The oxygen adsorption which was measured after three hours and finally after six days was as follows:

3 hours: a = 132, b = 155, c = 166, d = 249.

6 days: a = 503, b = 505, c = 513, d = 607

cc of oxygen respectively.

The experiment teaches us that the alkaline auto-oxidation of humus involves two distinct processes: 1. the activation by the alkali whereby the reduction potential is increased through an intramolecular rearrangement consisting, probably, in an intramolecular oxidation-reduction (since alkali alone produces a great increase in acids and acidoids, cf. system A 1 and C 1 table 29); and 2. the union with oxygen. The latter proceeds much faster in the activated humus, especially in the boiled system. The oxygen absorption of this system leads the others by about 100 cc even after six days.

This reminds us of the alkaline activation of glucose observed by CLIFTON and ORT (1930) who studied the reduction potential of the sugar as a function of the OH ion concentration. They report an enormous increase in the activity with increasing pH. The rate of activation was also greatly influenced by the temperature.

In another experiment the humus was oxidized in N. 0.25, N. 1, N. 2, N. 4, N. 8 and N. 16 solutions of NaOH. The evacuated flasks containing humus and solution stood over night in the constant temperature room before the oxygen was added. The results of the experiment are shown graphically in fig. 31. At the end of 14 days the systems had absorbed from 2.6 (N. 0.25) to 8.4 (N. 16) liters of oxygen on the basis of 100 grams humus. The concentration of alkali has a considerable effect up to N. 8. At N. 16 the results are anomalous in that the absorption is at first lower than that of any of the other systems whereas at the end of 14 days the N. 16 system shows the greatest absorption. The reason for this anomaly lies apparently in the powerful dehydration of the humus (or, perhaps, in its failure to hydrate). This was evident from the fact that the humus did not immediately solvate in the N. 16 alkali

solution but formed »curds» separated by a colorless solution. After some shaking the system assumed, however, a homogenous appearance.

The curves show a very rapid absorption of oxygen during the first 24 hours but once the activated form of humus has become saturated with oxygen the additional oxidation becomes an almost

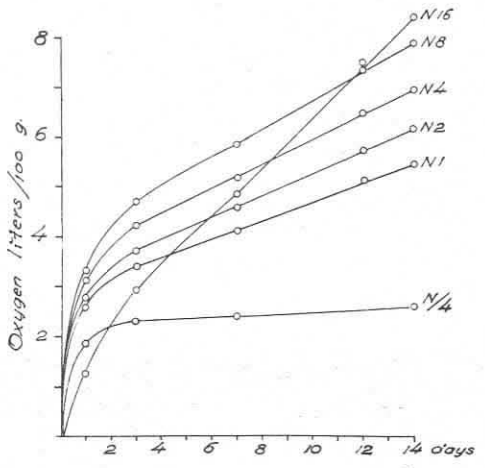



Fig. 31. The absorption of oxygen by a beech leave humus in N. 0.25 to N. 16 NaOH solutions.

linear function of time. It would be interesting to find out how long this linear relationship would continue and what the products would be after a prolonged oxidation. Would all the colloidal acidoids be broken up into acid-soluble $\beta$-acids, simple organic acids and carbon dioxide? Is the ratio carbon dioxide evolved to oxygen consumed as high in the early, rapid oxidation as in the later, slow oxidation?

The first question cannot now be answered but an experiment has been started which is intended to go for a long time, one year or more.

To find an answer to the second question three flasks, a, b and c, containing 10 grams of the humus an 200 cc N. 1 NaOH were evacuated. Flask a was immediately filled with oxygen and shaken for 8 days, flask b stood 8 days and was then filled with oxygen and shaken for three hours whereas flask c received no oxygen. The oxygen absorption was 17.83 m.mols in a and 6.69 m.mols in b. The carbon dioxide was then determined with the following results: a = 9.27, b = 3.25, and c = 1.52 millimols. By subtracting the carbon dioxide in c from that found in a and b we get the carbon dioxide due to the absorbed oxygen: a = 7.75 and b = 1.73

m.mols. This gives us a $CO_2/O_2$ ratio for the first three hours equal to $1.73/6.69 = 0.26$ and for the eight day period equal to $7.75/17.83 = 0.44$. If we subtract the oxygen consumed in b from that of a, and make the corresponding subtraction with respect to carbon dioxide we get the $CO_2/O_2$ ratio after the first three hours to the end of the eighth day. This gives us $6.02/11.14 = 0.54$, a ratio which is more than twice as great as that of the initial three hours.

Conclusion: the initial rapid oxidation of the alkali activated humus leads primarily to the formation of carboxylic acids and acidoids whereas the subsequent slow oxidation leads mostly to the production of carbon dioxide. The partial oxidation of the litter to humus acidoids increases the stability. *Humus acidoids must therefore accumulate in the soil to a level at which their decomposition balances the yearly increments.*

For a systematic study of auto-oxidation in alkaline solutions we selected the four vertical samples ($V_1F_1$, $F_2$ & H) of the Häggbygget podzol, the Annerstad high-moor sample II (20—30 cm), and the Ca-saturated Mölner moor sample I (cf. Part III).

The samples were given a pretreatment as follows:

30 grams (dry basis) were washed on the filter with N. 0.05 HCl until free from Ca, and then with water until a filtrate of 3 liters was obtained. The filtrates were examined with respect to the amount of organic matter and its base binding capacity ($\alpha$- and $\beta$-acids) as previously explained. Table 32 gives the results together with the acidoid content (by titration to pH 7 in N. KCl solution), the $pH_u$, and the N-content of the electrodialysed samples of the original materials. The titration curves are shown in fig. 33 A.

Table 32 brings out one very significant fact. It will be noted that the Mölner, which in its natural Ca-saturated condition is granular, compact and nondispersible, yields large amounts of acids (dark colored $\beta$-acids) when leached after desaturation. This sample, which possesses the highest acidoid content of any humus examined by us, is very dispersible in the unsaturated condition and yields very dark colored solutions, both anode and cathode, when electrodialysed. It is impossible to desaturate the Mölner without considerable losses. Other samples of humus formed under conditions of a high base status behave in the same way. This we ascribe to the low equivalent weight of the humus acidoids (= high polarity = short molecular chains). If the »sour» humus of the podzolized regions developed as high an acidoid content it would, unsaturated as it is, largely pass into solution and color the water of our streams and rivers black. The black rivers of South America owe their color to humus acidoids formed under conditions of a high base status followed by intense leaching. The tshernozems, or black soils of the steppes, rapidly lose their humus when degraded by leaching. The acidoids of »mild» humus are much

stronger than the acidoids of »sour» humus and must, when unsaturated, exert a much greater solvent action. If the former were formed under the conditions of a low base status podzolization would be a more serious problem than it is.

Table 32 also shows how the amount of soluble organic matter decreases from V to H in the podzol profile and how the capacity to bind base per 100 grams of this organic matter ($y/x \cdot 100$) increases in the same direction. Note also how the ratio $A(\text{pH } 10)/A(\text{pH } 7)$ decreases from V (were it is very wide) to H, and that this ratio is lowest in the Mölner, i.e., in the most highly oxidized humus. We shall presently see that artificial oxidation leads to the same result, i.e., an increase in the strong acidoid groups (carboxyl) at the expence of the weak acidoid groups (phenol hydroxyl).

Table 32 is chiefly intended for comparison with tables 33 a and b.

The residues from the pretreatment were then treated as follows:

A quantity of each sample equal to 22 grams of organic matter was placed together with 400 cc N/1 NaOH in vacuum flasks of 2 liters capacity. The flasks were then evacuated and filled with oxygen gas and placed in a constant temperature room at 20° C. At intervals of two days the volume of oxygen required to equalize the pressure in the flasks was passed through and measured in a 500 cc gas burette. The flasks were shaken in a machine seven hours daily for fourteen days. The absorption of oxygen is shown in fig. 32 A.

At the end of the treatment the carbon dioxide was determined as already described and the acidified contents of the flasks were filtered and washed until 2200 cc of filtrate were obtained. The $\alpha$ and $\beta$-acids were determined in the filtrate as before and the residues were electrodialysed and the acidoid and nitrogen content determined. The results are given in tables 33 a and b and the titration curves are shown in fig. 33 B.

The absorption of oxygen was most rapid in the beginning (cf. fig. 32 A) and was greatest in the $F_2$ sample which adsorbed 7805 cc or 324 millimols per 100 grams organic matter. The absorption was smallest in the Annerstad sample due, possibly, to a relatively high proportion of cellulose in the highmoor humus (cellulose absorbs oxygen slightly, cf. below) or to a protection by waxy substances. The low absorbing power of the Mölner humus may be ascribed to the fact that this humus is already highly oxidized.

The carbon dioxide evolved is somewhat smaller than one half the equivalent of oxygen absorbed except in the case of the Mölner where it exceeds one half of the oxygen.

The »respiration» of the samples has led to a great increase in soluble organic matter and in $\alpha$ and $\beta$-acids, the increase being

Table 32. *The amount of soluble organic matter and its acidity (a- and β-acids), and the acidoid (A) and nitrogen content and $pH_u$ of the original samples of the Häggbygget podzol (V, $F_1$, $F_2$ & H) and of the Annerstad and the Mölner peat.*

| Sample | In filtrate from 100 g.: | | | In 100 g. org. matter: | | | | $pH_u$ in N.KCl |
|---|---|---|---|---|---|---|---|---|
| | Organic matter (x) | a & β acids (y) | $\frac{y}{x} \times 100$ | A(pH 7) | A(pH 10) | $\frac{A(pH\ 10)}{A(pH\ 7)}$ | N | |
| | g. | m.e. | m.e. | m.e. | m.e. | | % | |
| V Häggbygget | 10.9 | 18.5 | 170 | 62 | 230 | 3.74 | 1.05 | 2.40 |
| $F_1$ » | 4.7 | 14.5 | 309 | 87 | 242 | 2.78 | 1.24 | 2.29 |
| $F_2$ » | 3.3 | 10.6 | 322 | 127 | 276 | 2.17 | 1.81 | 2.16 |
| H » | 2.5 | 11.4 | 456 | 157 | 315 | 2.01 | 2.03 | 2.05 |
| Annerstad ..... | 1.4 | 8.3 | 593 | 121 | 220 | 1.82 | 1.20 | 2.05 |
| Mölner ..... | 10.8 | 58.7 | 544 | 319 | 423 | 1.33 | 3.94 | 1.64 |

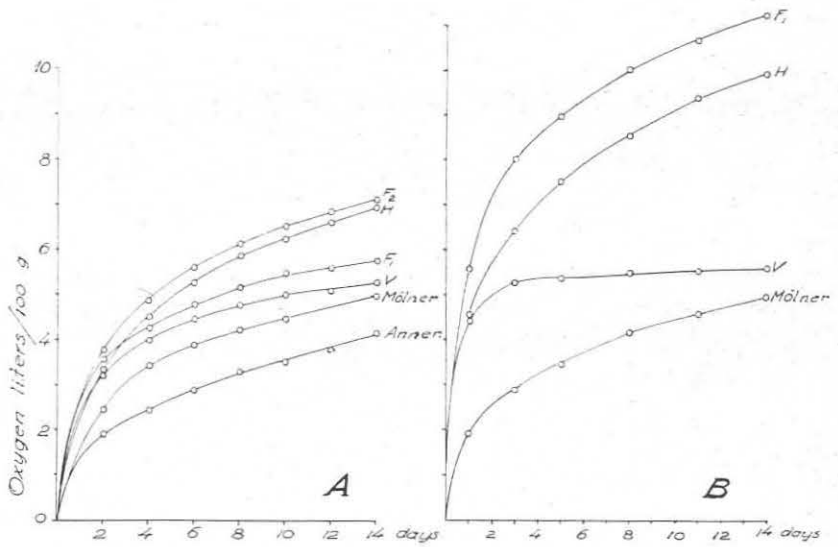



Fig. 32. The absorption of oxygen (A) by the whole samples in table 33 a, and (B) by the »lignin» samples in table 35 a.

greatest in the Mölner. The distribution between the *a* and *β* fractions is, however, here different from that resulting from peroxide as shown in table 29 series C. The oxygen being less drastic leaves a much greater proportion of the *β*-fraction. Expressed in terms of acidity (m.e.) this fraction is over twice as great as the *a*-fraction in every sample, and is over three times as great in the case of the Mölner.

Table 33 a. *The products of a partial oxidation in alkaline solution by oxygen of the leached samples (table 32).*

| Sample | Per 100 g. original organic matter: | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $O_2$ consumed[1] | $CO_2$ evolved (x) | Soluble organic matter: | | Insol. residue[2] | $a$ | $\beta$ | $A$ | Increase[3] $a+\beta+A$ (y) | $\frac{CO_2}{a+\beta+A}$ $\frac{x}{y}$ |
| | | | $a$ | $\beta$ | | | | | | |
| | m.mols | m.mols | g. | g. | g. | m.e. | m.e. | m.e. | m.e. | |
| V Häggbygget | 240 | 112 | 16.6 | 18.5 | 69.2 | 107 | 164 | 52 | 261 | 0.43 |
| $F_1$ » | 262 | 118 | 16.0 | 27.3 | 64.1 | 101 | 211 | 71 | 296 | .40 |
| $F_2$ » | 324 | 153 | 17.7 | 30.8 | 55.9 | 115 | 241 | 87 | 316 | .48 |
| H » | 318 | 150 | 18.5 | 31.3 | 60.0 | 113 | 238 | 111 | 305 | .49 |
| Annerstad ... | 189 | 82 | 17.5 | 23.7 | 62.3 | 111 | 157 | 104 | 251 | .33 |
| Mölner ..... | 206 | 115 | 12.9 | 40.2 | 46.4 | 79 | 274 | 133 | 167 | .69 |

[1] 1 liter $O_2$ at 20° C and 760 mm Hg = 41.56 m.mols.
[2] Figures not exact due to loss on transfer.
[3] By subtracting the acidoids of the original samples (table 32).

Table 33 b. *The capacity of the acids and acidoids (in table 33 a) to bind base, and the $pH_u$ and nitrogen content of the electrodialysed residue.*

| Sample | In 100 g. organic matter: | | | | | | $pH_u$ in N.KCl |
|---|---|---|---|---|---|---|---|
| | $a$ | $\beta$ | $A$(pH 7) | $A$(pH 10) | $\frac{A(pH\ 10)}{A(pH\ 7)}$ | N | |
| | m.e. | m.e. | m.e. | m.e. | | % | |
| V Häggbygget | 645 | 886 | 75 | 102 | 1.36 | .67 | 2.15 |
| $F_1$ » | 631 | 773 | 111 | 161 | 1.45 | .96 | 2.05 |
| $F_2$ » | 650 | 782 | 156 | 238 | 1.53 | 1.64 | 1.98 |
| H » | 611 | 760 | 185 | 285 | 1.54 | 1.96 | 1.91 |
| Annerstad ..... | 634 | 662 | 167 | 255 | 1.53 | .97 | 1.96 |
| Mölner ..... | 612 | 682 | 297 | 404 | 1.36 | 4.05 | 1.65 |

The increase in the acidity due to the oxidation mounts to a maximum of 316 m.e. in $F_2$. In the already highly oxidized Mölner the increase is only 167 m.e. The large amount of $\beta$-acids (274 m.e.) of this sample has, therefore, largely been formed at the expence of the original acidoid content which has decreased from 319 to 133 m.e. The ratio of carbon dioxide to the increase in acidity is smaller than 0.5 in every case except that of the Mölner in which it is 0.69. The latter which is already highly oxidized yields a relatively greater amount of carbon dioxide.

If we now turn our attention to table 33 b we find that the base binding capacities per 100 grams of the $a$ and $\beta$-fractions are about the same as that of systems 2 and 3 in series C in table 30 to which

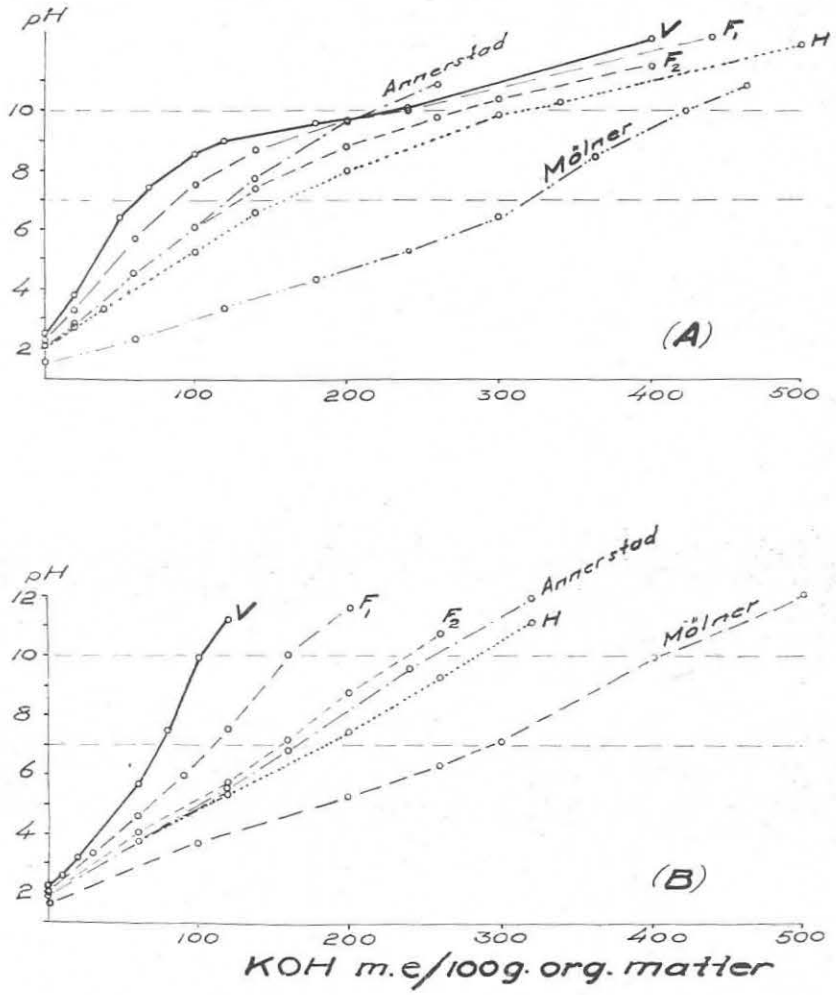



Fig. 33. The titration curves (A) of the unoxidized samples (table 32), and (B) of the auto-oxidized samples (table 33 b).

only moderate amounts of hydrogen peroxide (5 to 10 cc) were added. The capacities of the *a* fractions are remarkably constant in that they vary only between 611 and 650 m.e. The *β*-acids of the V sample possess the highest capacity (886 m.e.). The *β*-acids of the $F_1$, $F_2$ and H samples all have capacities which vary only between 760 and 782 m.e.

The capacities of the oxidized acidoids to bind base show a very significant trend (cf. also fig. 33 A and B). Thus the capacities at pH 7 have increased in every case except that of the Mölner where-

as the capacities at pH 10 have decreased in every case except that of the Annerstad, the decrease being over 50 percent in the V sample. The ratios $A$(pH 10)/$A$(pH 7), which in the original samples varied between 3.74 and 1.33, have been decreased by the oxidation to values which are nearly the same for all the samples, and of an order of magnitude which approximates that of the original Mölner which has remained practically unchanged. This means that auto-oxidation in an alkaline medium (or merely under the conditions of a high base status in nature e.g. the Mölner) leads to an increase of the strong acidoid groups (carboxyl) at the expense of the weak acidoid groups (phenol hydroxyl). We look upon this as an important clue to the constitution of certain plant materials and to the nature of humus (cf. general discussion).

The nitrogen content is lower in the oxidized podzol samples. The decrease is 0.38, 0.28, 0.17 and 0.07 % in the V, $F_1$, $F_2$ and H samples respectively. This does not mean that the oxidized complex becomes poorer in nitrogen — we believe the contrary to be true — but rather that some of the component with which the nitrogen is associated, i.e., the lignin, has been solvated and lost (see below).

It remains to point out that the $pH_u$ of the oxidized samples show the same relationship to the acidoid content as before the oxidation. The values fit the curve in fig. 40 very well.

### b. The acid »lignin» fraction.

It is now generally believed that lignin constitutes the major part of the plant materials from which humus acidoids are formed. Cellulose and other carbohydrates are relatively easely decomposed whereas lignin (or lignin-like products) tend to accumulate in the humus (WAKSMAN). Now if the humus acidoids are primarily derived from the lignin complex we ought to find a higher acidoid content in the residue obtained by an acid hydrolysis of humus whereby the hemicellulose and cellulose are dissolved and eliminated. An alkaline auto-oxidation of such residues ought to yield products possessing the highest capacities to bind base. We therefore decided to subject some of our samples to an acid hydrolysis and to study the behavior of the residues. This study includes the Häggbygget V, $F_1$, and H samples, the Mölner humus and a sample of wheat straw.

Samples of 100 grams were extracted with ether and digested in one liter 72 percent sulphuric acid for 18 hours at a temperature between 16 and 18° C. They were then placed in glass cylinders of 20 liters capacity and diluted and decanted several times, the usual boiling after dilution being omitted. They were then thoroughly

washed by means of a battery of Pasteur-Chamberland filters and finally dried in the air. The percentage of organic matter (= loss on ignition) recovered in the residues were as follows: V = 47.6, $F_1$ 60.5, H = 77.1, Mölner = 80.6, and straw = 31.1.

The »lignin» residues were divided into two parts one of which was electrodialysed directly and examined with the results shown in table 34 and fig. 34 A, whereas the other part was first subjected to an alkaline auto-oxidation (cf. fig. 32 B). The oxidized samples were then examined by the same methods as in the case of the original samples (cf. tables 33 a and b) with the results given in tables 35 a and b and in fig. 34 B.

Table 34. *The acidoid, nitrogen and sulfate content and the $pH_u$ of the »lignin» residue from the 72 % sulphuric acid treatment.*

| »Lignin» from | Per 100 g. organic matter: | | | | | $pH_u$ in N. KCl |
|---|---|---|---|---|---|---|
| | $A$(pH 7) m.e. | $A$(pH 10) m.e. | $\frac{A(\text{pH 10})}{A(\text{pH 7})}$ | N % | $SO_3$ % | |
| V Häggbygget ..... | 111 | 278 | 2.50 | 1.79 | 0.34 | 1.91 |
| $F_1$ » ..... | 177 | 389 | 2.20 | 2.03 | .38 | 1.74 |
| H » ..... | 234 | 453 | 1.94 | 2.29 | .60 | 1.62 |
| Mölner ..... | 368 | 512 | 1.39 | 3.82 | 1.87 | 1.45 |
| Wheat straw..... | 74 | 230 | 3.11 | 2.14 | n. d. | 2.21 |

Table 35 a. *The products of a partial oxidation in alkaline solution by oxygen of the »lignin» samples (table 34).*

| »Lignin» from | Per 100 g. original organic matter: | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $O_2$ consumed | $CO_2$ evolved | Sol. org. matter | | Insol. residue | $\alpha$ | $\beta$ | $A$ | Increase[1] $\alpha+\beta+A$ | $\frac{CO_2}{\alpha+\beta+A}$ |
| | | | $\alpha$ | $\beta$ | | | | | | |
| | m.mols | m.mols | g. | g. | g. | m.e. | m.e. | m.e. | m.e. | |
| V Häggbygget . | 231 | lost | 12.5 | 32.9 | 57.7 | 100 | 282 | 144 | 415 | — |
| $F_1$ » . | 466 | 208 | 12.8 | 39.7 | 51.4 | 109 | 350 | 152 | 434 | 0.48 |
| H » . | 391 | 190 | 11.5 | 37.1 | 52.7 | 100 | 330 | 167 | 363 | 0.52 |
| Mölner ..... | 206 | lost | 6.8 | 48.8 | 47.7 | 44 | 370 | 170 | 220 | — |
| Wheat straw ... | 178 | 62 | 4.8 | 13.0 | 50.5 | 48 | 54 | 101 | 129 | 0.48 |

[1] By subtracting original acidoids (table 34).

The absorption of oxygen is greatest (11.25 liters per 100 g) in the $F_1$ sample thus indicating that the »lignin» complex is most active in slightly humified materials. The »lignin» complex of the V sample appears to be very stable once the activated form has become saturated with oxygen, the secondary, slow oxidations

Table 35 b. *The capacity of the acids and acidoids (table 35 a) to bind base, and the nitrogen content and $pH_u$ of the electrodialysed residue.*

| »Lignin» from | In 100 g. organic matter: | | | | | | $pH_u$ in N. KCl |
|---|---|---|---|---|---|---|---|
| | $\alpha$ m.e. | $\beta$ m.e. | $A$(pH 7) m.e. | $A$(pH 10) m.e. | $A$(pH 10) / $A$ pH 7 | N % | |
| V Häggbygget | 800 | 857 | 249 | 362 | 1.45 | 1.98 | 1.60 |
| $F_1$ » | 852 | 882 | 295 | 425 | 1.44 | 2.34 | 1.59 |
| H » | 870 | 889 | 317 | 498 | 1.57 | 2.46 | 1.53 |
| Mölner ..... | 641 | 758 | 357 | 463 | 1.30 | 3.74 | 1.59 |
| Wheat straw .. | 1000 | 415 | 201 | 311 | 1.55 | 2.62 | 1.91 |

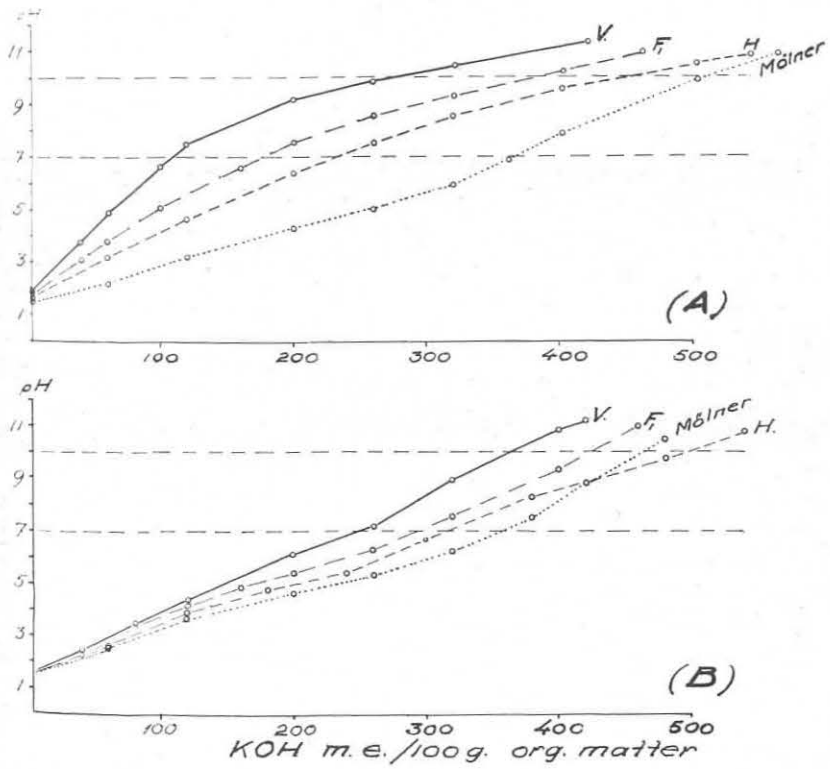



Fig. 34. The titration curves (A) of the unoxidized »lignin» samples (table 34), and (B) of the auto-oxidized »lignin» samples (table 35 b).

being very small in this sample. The already, in nature, highly oxidized Mölner shows no increase in the capacity of the »lignin» complex to absorb oxygen (comp. fig. 32 A). The Mölner has orig-

inally a high »lignin» content. The absorption of oxygen by the straw lignin is shown in fig. 36.

If we compare table 34 with table 32 we find a very great increase in the acidoid content at pH 7 in samples V and $F_1$ (nearly 100 %), a considerable increase in the H sample (about 50 %) and a small increase in the Mölner. These increases depend, no doubt, largely on the percentage increase of the »lignin» component in which the acidoid properties apparently reside. But there are many other possible factors which might have influenced the capacity to bind base: The sulphuric acid might have destroyed or caused some acidoid groups to pass in solution or it might have created new such groups by decomposing the esters of lignin and carbohydrates which many assume to exist (HÄGGLUND 1938) and, finally, it might have introduced a certain number of sulphonic acid groups. That this number cannot be large is indicated by the amounts of total $SO_3$ found in the samples (cf. table 34). The unoxidized straw lignin possesses only moderate capacity to bind base at pH 7. The straw lignin, which is included here merely for the sake of comparison, will be discussed in detail in connection with table 36.

The acidoid content at pH 10 shows, by the same comparison, a smaller increase than at pH 7 in the V and $F_1$ samples (cf. decrease in the ratios $A$ (pH 10)/$A$ (pH 7)) as if some of the weaker acidoid groups had originally belonged to the carbohydrate fraction or else had been destroyed.

As to the nitrogen we note a considerable increase in the V and $F_1$ samples. This indicates that the nondialyseable nitrogen belongs chiefly to the »lignin» fraction.

The $pH_u$ has decreased and it has done this out of proportion to the increase in acidoids. Hence these values fall below the curve in fig. 40 (x marks). This might be due to an introduction of a few sulphonic acid groups or it might be the result of the fact that the acidoid content has been increased more than the nitrogen content.

If we now compare table 35 a with 33 a we find it noteworthy that the »lignin» samples yield relatively much more $\beta$-acids than $a$-acids as compared to the original samples. (Note that straw lignin yields much less $\beta$-acids.) The conclusion seems to be that the carbohydrates are the mother substance of most of the $a$-acids whereas some modified form of lignin is the mother substance of the $\beta$-acids. We note further that there is an absolute increase (from 111 to 144 m.e.) of acidoids in the V-sample and that the total acidity ($a+\beta+A$) has increased much more by the oxidation of »lignin» than by that of the original samples. The ratio $CO_2/a+\beta+A$ seems to be about the same in both series of samples. (We regret that two of the carbon dioxide determinations were lost by foaming.)

Table 35 b should be compared with table 33 b as well as with table 34. The most striking fact here is the enormous increase in the acidoid content of the three podzol samples, two of which ($F_1$ and H) have reached a value of the order of magnitude of that of the original Mölner or about 300 m.e./100 g. The experiment shows conclusively that the acidoid groups belong to the »lignin» fraction.

The capacities to bind base at pH 10 show a much smaller increase. This reflects itself in the ratios $A$(pH 10)/$A$(pH 7) which again seem to have reached a limiting value of about 1.5. The inevitable conclusion seems to be that the carboxyl groups are formed at the expence of the phenol hydroxyls, probably by rupture of the benzene rings at the position of the latter groups as when the naphthols are oxidized (see below).

The nitrogen content of the podzol samples has been increased by the oxidation of the »lignin». In the case of the original samples it was, it will be remembered, somewhat decreased. Our explanation of these apparently contradictory results is the following: The original samples contained an insoluble and inactive carbohydrate »skeleton». During the alkaline oxidation a large part of the »lignin» is dissolved, forming $\beta$-acids etc. The final product contained, therefore, less »lignin» and relatively more carbohydrates. In the case of the »lignin» samples the mere loss of »lignin» should not affect the composition of the residue since it is all »lignin». Hence the nitrogen, if it belongs to the lignin fraction, as we believe it does, should not decrease. The fact that the nitrogen in the oxidized »lignin» samples has increased (in spite of the fact that alkali tends to spit off some ammonia) leads to the conclusion, previously made, that nitrogen increases the stability of the humus acidoids and must, therefore, tend to accumulate. The same seems to apply to the ligno-sulphonic acids which are less soluble in combination with aromatic amines (HÄGGLUND).

The $pH_u$ values of the oxidized »lignin» samples are also, with the exception of the Mölner and the straw lignin, »abnormally» low (cf. fig. 40).

The curves in fig. 33 and 34 clearly reveal the most fundamental change, i.e., in the acidic condition, brought about by the oxidation. This change is, however, more strikingly illustrated by combining the curves (unoxidized and oxidized) pairwise as in fig. 35. We note that the curves intersect, the intersection occuring at a higher pH for the »lignin» than for the original samples. We note also that the difference between the curves becomes less with the age of the material, i.e., with the increase in strong acidoids. The Mölner curves do not intersect. The marked inflection between the strong and the weak acidoids, so evident in the original V sample, vanishes with age and with oxidation. Conclusion: *The aerobic*

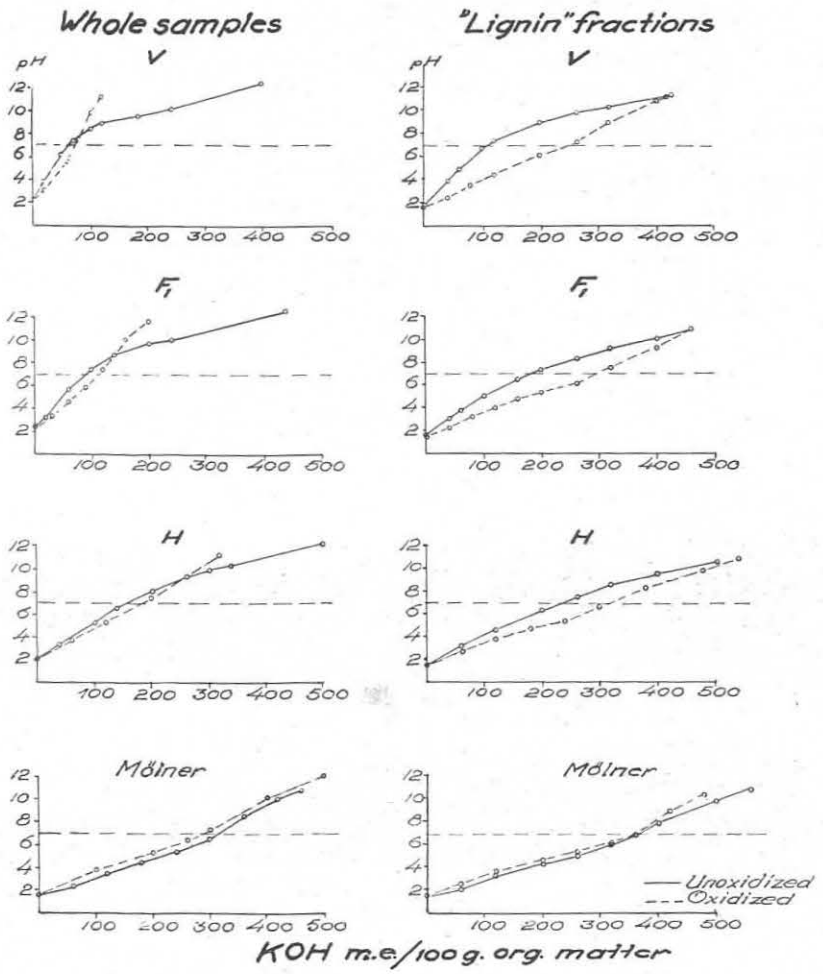



Fig. 35. The same curves as in fig. 33 and 34 shown in pairs of unoxidized and oxidized.

*formation of humus acidoids consists essentially in an oxidation of the lignin to ligno-carboxyl acidoids.* We believe this oxidation to be associated with the fixation of nitrogen to be discussed below.

### c. Lignin, protein and cellulose.

The acidic properties of humus are assumed by WAKSMAN (1938) to be associated with the ligno-nitrogenous fraction. This assump-

tion is supported, among other things, by the fact that humification, especially at a high base status, leads to a decrease in cellulose and an increase in the lignin-like fraction and nitrogen at the same time as the acidoid content also increases. WAKSMAN looks, however, upon the ligno-nitrogenous complex as a compound of lignin and protein (his »humus-nucleus») of the nature of the ligno-protein precipitates studied by WAKSMAN and IYER (1932). The high base exchange capacity ascribed to their compounds is accounted for on the assumption of a linkage between the ammino group of the protein and the carbonyl group of the lignin of the nature of a Schiff's base:

$$R \cdot CO + H_2N \cdot R \cdot COOH \rightarrow R \cdot C : N \cdot R \cdot COOH + H_2O$$

The increase in the power to bind base reported by WAKSMAN and IYER is contrary to the findings of MATTSON (1932) who found a lower capacity in his protein humates than in the original humus. Below the I. E. P. of the protein it entered as a cation and replaced some of the metal cations exactly as one would expect. WAKSMAN means, however, that humus, in which the lignin is already linked to protein, cannot behave in the same way as free lignin. TIURIN and LEYN (1940) deny, however, that there is any increase in the exchange capacity of such precipitates. The capacity is always intermediate between that of the individual components. WAKSMAN and IYER erred by failing to »take into consideration the fact that the exchange capacity of lignin is many times increased by a treatment with weak alkali».

In order to throw new light on the ligno-protein complex we decided to include such a complex in our study and selected for this purpose the acid lignin of wheat straw and casein. For comparison we also studied the oxidation and titration of the original straw and for the sake of completion we also included another major constituent of plants, namely cellulose. The latter is a sample of sulphite (wood) cellulose of the kind now used in Sweden as a cattle feed. The straw lignin was prepared as described in the preceding chapter. The casein lignate was isoelectrically precipitated (at pH 4) by mixing 20 grams of lignin, dispersed in one liter with NaOH, with 5 grams of casein (HAMMARSTEN), dissolved in one liter with HCl. The precipitate was washed free from Cl and dried at 50° C.

The casein lignate (the whole sample) and samples of 20 grams of straw, lignin and cellulose and a sample of 5 grams of casein, all finely powdered, were then studied with respect to their alkaline auto-oxidation. The casein did not absorb a measurable trace of oxygen. The absorption of oxygen by the other samples is shown in fig. 36 where the absorption is expressed per 100 grams (in the case of the casein lignate per 100 grams lignin).

The lignin absorbed in 12 days 4275 cc oxygen while the casein lignate absorbed 4305 cc. The increase is insignificant and shows that the casein does not affect the oxidation of lignin as it might be expected to do if it were tied up to an aldehyde carbonyl of the lignin. The straw absorbed, in proportion to its lignin content, a

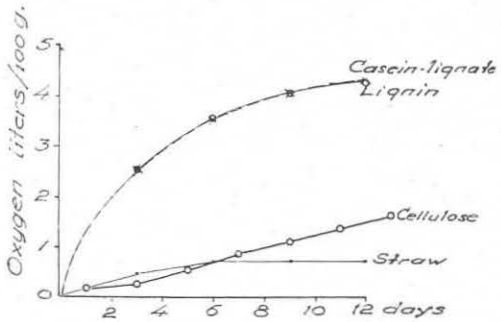



Fig. 36. The absorption of oxygen by the samples in table 36.

small amount (725 cc) of oxygen. This might be ascribed either to an incrustation of the lignin or to a chemical union with the carbohydrates.

The cellulose gives a different curve. The absorption is appreciable but the initial rapid oxidation is here absent. The oxidation might be due to sugar or to residual lignin or both (filter paper was found to absorb very little oxygen). The products of the oxidation of the cellulose were practically all soluble acids and carbon dioxide (25 m.mols $CO_2$ from 68 m.mols $O_2$ absorbed). The acidoids of the cellulose were only slightly increased by the oxidation (cf. table 36). Cellulose, apparently, does not contribute to the formation of humus acidoids, at least not by direct oxidation. We

Table 36. *The acidoid and nitrogen content and $pH_u$ of wheat straw, straw lignin, casein lignate and wood cellulose before and after an alkaline auto-oxidation.*

| Sample | Per 100 g. organic matter: | | | | $pH_u$ in N. KCl |
|---|---|---|---|---|---|
| | *A*(pH 7) m.e. | *A*(pH 10) m.e. | *A*(pH 10) / *A*(ph 7) | N % | |
| Straw | 21 | 87 | 4.14 | 0.61 | 3.06 |
| » oxidized | 35 | 59 | 1.69 | 0.51 | 2.86 |
| Lignin | 74 | 230 | 3.11 | 2.14 | 2.21 |
| » oxidized | 201 | 311 | 1.55 | 2.62 | 1.91 |
| Casein lignate oxidized | 190 | 283 | 1.49 | 4.79 | 2.65 |
| Cellulose | 16 | 34 | 2.12 | 0.03 | 1.94 |
| » oxidized | 18 | 32 | 1.76 | 0.01 | 2.32 |

have, perhaps, here an explanation why cellulose does not, like lignin, accumulate in the soil: Cellulose does not, like lignin, form relatively stable, nitrogen containing colloidal acids.

Table 36 shows the acidoid and nitrogen content of the unoxidized and oxidized samples. Fig. 37 shows the titration curves.

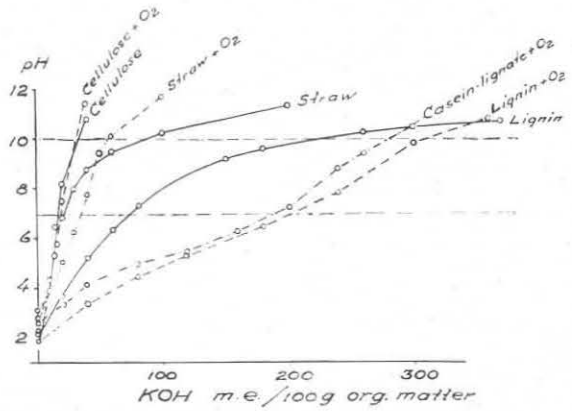



Fig. 37. The titration curves of the samples in table 36.

The soluble products of the oxidation were studied only in the case of the lignin which, for this reason was included in table 35 a. The most noteworthy thing there is the small amounts of $\beta$-acids and the fact that the straw lignin shows, like the V sample, an absolute gain in acidoids as a result of the oxidation. The »unmodified» lignin is apparently more stable, even when oxidized, than the modified humus »lignin».

Table 36 and fig. 37 give us a good picture of what happens when straw, lignin and cellulose undergo an alkaline auto-oxidation. As far as the straw and lignin are concerned the picture is much the same as that of the »lignin» samples in the preceding chapter. We note the enormous base binding capacity at high pH (above 9) of the unoxidized straw and lignin in which the number of phenol hydroxyl must be very large. In the case of the cellulose there is no such capacity to bind base at high pH and the oxidation has produced very slight changes in the titration curve. The slight increase in the acidoid content at pH 7 and the slight decrease at pH 10 might be ascribed to residual lignin.

The nitrogen content in the lignin has been somewhat increased by the oxidation, which again points to a greater stability of the nitrogenous complex. In the straw we note a slight decrease of nitrogen due, undoubtedly, as already pointed out, to a greater loss of lignin than carbohydras during the treatment.

The $pH_u$ values of the straw and lignin fit the curve in fig. 40

very closely. The $pH_u$ values of the cellulose, especially that of the untreated sample, are abnormally low. This must be ascribed to the presence of ligno-sulphonic acid groups from the sulphite cooking.

The properties of casein lignate are best brought out by comparing its curve with that of the oxidized lignin in fig. 37. We note that the casein lignate binds less base than the free lignin, the percentage decrease being considerable below the I. E. P. of the protein (pH 4.8) where it functions as a base. This reflects itself also in an abnormally high $pH_u$ (cf. also fig. 40).

The capacity to bind base at pH 7 is much greater in the oxidized casein lignate than in the unoxidized lignin. From this fact we can easely understand how anyone, working with the methods employed by WAKSMAN and IYER, might arrive at an erroneous conclusion with respect to the function of the protein. The proteins combine, apparently, with lignin in the same manner in which they combine with the natural humus acidoids as established by MATTSON in his study of their isoelectric precipitates.

## The influence of different bases on auto-oxidation.

The absorption of oxygen was studied in the presence of an excess of $CaCO_3$, $Mg(OH)_2$, $Ca(OH)_2$ and $Ba(OH)_2$ as follows:

Samples of the Häggbygget podzol profile materials, the Annerstad highmoor and the Mölner lowmoor humus corresponding to 20 grams organic matter were placed in the vacuum flasks (2 l.) and dispersed in 400 cc water containing 20 m. e. NaOH. To each system 300 m. e. of $CaCO_3$ or of one of the alkaline earth bases were then added as indicated in table 37. In the systems containing $CaCO_3$ three large test tubes, partly filled with 4 N. NaOH, provided for the adsorption of carbon dioxide. The flasks were then evacuated, filled with oxygen and shaken for two weeks, the absorbed oxygen being replaced and measured every other day as in the previous experiments. At the end the contents of the flasks were neutralized with HCl and washed on the filter with N. 0.05 HCl to remove the bases and then with water. The residues were then electrodialysed. The results obtained are shown in table 37. We include for comparision sample H in tables 33 a and b (base NaOH) and the untreated sample in table 32. Since nothing was added to produce sterile conditions some of the activity in the carbonate systems can be credited to microorganisms.

In the carbonate systems the absorption of oxygen is surprisingly great, reaching 206 m.mols in the V sample. It is lowest in the Mölner as might be expected since this humus has been formed under a high base status. It is lower in $F_1$ than in H but this might be due to the lower final pH in the former.

Table 37. *The absorption of oxygen in the presence of different bases and the effect on the acidoid and nitrogen content and on the $pH_u$.*

| Sample | Base | pH at the end | $O_2$ consumed m.mols | $A$ (pH 7) m.e. | $A$ (pH 10) m.e. | $\frac{A(\text{pH } 10)}{A(\text{pH } 7)}$ | N % | $pH_u$ in N. KCl |
|---|---|---|---|---|---|---|---|---|
| Annerstad ... | $CaCO_3$ | 7.50 | 50 | 121 | 217 | 1.79 | 1.13 | 2.13 |
| Mölner ..... | » | 7.37 | 40 | 300 | 419 | 1.40 | 3.88 | 1.68 |
| V Äggb. .. | » | 7.01 | 206 | 74 | 218 | 2.95 | 1.03 | 2.30 |
| $F_1$ » .. | » | 6.83 | 117 | 95 | 233 | 2.45 | 1.33 | 2.26 |
| F » .. | » | 7.46 | 139 | 150 | 300 | 2.00 | 1.88 | 2.06 |
| H » .. | $Mg(OH)_2$ | 9.09 | 186 | 184 | 304 | 1.65 | 1.96 | 1.80 |
| H » .. | $Ca(OH)_2$ | 12.08 | 202 | 228 | 349 | 1.53 | 2.03 | 1.81 |
| H » .. | $Ba(OH)_2$ | 12.72 | 285 | 229 | 356 | 1.55 | 1.95 | 1.78 |
| H » .. | NaOH | N.D. | 318 | 185 | 285 | 1.54 | 1.96 | 1.91 |
| H » .. | Untreated | — | — | 157 | 315 | 2.01 | 2.03 | 2.05 |

In the alkaline earth bases the absorption of oxygen increases with the solubility of the base and the pH of the system.

In the carbonate systems the acidoid capacity at pH 7 has, in general, been slightly increased whereas the capacity at pH 10 is somewhat decreased. The ratios $A(\text{pH } 10)/A(\text{pH } 7)$ are, therefore, mostly a little lower. The nitrogen content and the $pH_u$ have suffered slight changes in these systems.

In the alkaline earth bases the acidoid capacity at pH 7 shows a considerable increase in all of the three systems whereas at pH 10 only the Ca and Ba systems have registered an increase. The ratio $A(\text{pH } 10)/A(\text{pH } 7)$ is, however, lower and almost the same in all three. The lower capacities in the Na system is to be ascribed to a greater proportion of the nonacidic skeleton mentioned above. In the Na system the alkalinity was higher and the dispersing action of the alkali was greater, causing more $\beta$-acids to split off. Some (not all) of the dark colored $\beta$-acids are precipitated by Ca or Ba ions if the solution is rendered alkaline. In the alkaline earth systems the $\beta$-acids were, therefore, never completely dispersed, even if split off from the mother complex, and did not spontaneously disperse so readily when the system was acidified. We do not, however, claim that there is a sharp distinction between the $\beta$-acids and the colloidal acidoids nor that anything but an approximate fractionation is accomplished by filtration.

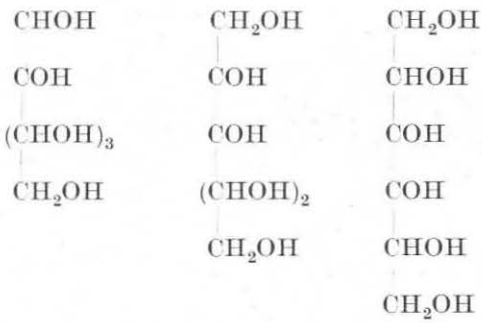
The nitrogen content of the alkaline earth series is in general a little lower than in the original H sample. The $pH_u$ values show here, as elsewhere, a definite relationship to the acidoid concentration (fig. 40).

## Oxidation of glucose.

To account for the low acidoid level in »sour» humus and for the high level in »mild» humus we have assumed that the rates of the two steps in the oxidation of organic matter, viz, the partial oxidation to carboxyl and the complete oxidation to carbon dioxide and water, are differently affected by the reaction of the medium in such a way that a high pH favors the oxidation to carboxyl more than it favors the oxidation to carbon dioxide. The experiment described in table 29 support this assumption.

In order to find out if this likewise applies to the oxidation of simple and physiologically important substances like the sugars we decided to study the oxidation of glucose with hydrogen peroxide by the same method employed for humus. Five grams of glucose, 5 cc of 30 % hydrogen peroxide and 15 cc of water (or acid or alkali solution) together with the various electrolytes named in table 38 were placed in the vacuum flasks which were then evacuated and kept at 37° C for one week. The carbon dioxide and the acidity formed by the reaction were determined and the amounts due to the added electrolytes were deducted. Table 38 gives the results.

The oxidation of the sugars in alkaline solution has been most extensively studied by NEF (1914) and later by EVANS (1929). NEF assumes that the alkali causes the hexoses to form 1—2, 2—3 and 3—4 dienols:




These dienols then split at the double bond giving rise to fragments which contain divalent carbon and which therefore, are very reactive. They may react with each other and they may polymerize to more stable compounds. One fragment may be oxidized and the other reduced thus forming acids in the absence of oxygen or any other hydrogen acceptor. They readily combine with oxygen to form a great number of organic acids, from carbonic and formic to those containing several carbon atoms. The products vary with the conditions such as concentration of alkali, oxidation potential, temperature, time, and the presence and nature of various catalyzers.

Thus alkaline silver oxide yields mostly carbonic, formic and oxalic acids (comp. theory of STIEGLITZ cited in Part III).

WITZEMANN (1920) studied the oxidation of glucose by hydrogen peroxide and found that the presence of disodium phosphate brings about a complete oxidation to carbon dioxide and water. We included, therefore, mono- and diphosphates among the chemicals added to our systems.

Table 38. *Acids and carbon dioxide formed by the oxidation of glucose by hydrogen peroxide.*

Each system contained:
5 grams glucose,
5 cc 30 % hydrogen peroxide,
15 » water

| Treatment | Acids m.e. | $CO_2$ m.e. | $\frac{Acids}{CO_2}$ |
|---|---|---|---|
| 5.0 m.mol HCl ..... | 3.8 | 2.9 | 1.31 |
| 0.5 » » ..... | 9.8 | 7.5 | 1.31 |
| None ..... | 19.9 | 13.8 | 1.44 |
| 19.85 m.mol $CaCO_3$ ... | 26.5 | 14.3 | 1.85 |
| 17.27 » MgO ..... | 31.1 | 6.7 | 4.64 |
| 40.0 » NaOH ... | 39.6 | 5.6 | 7.07 |
| 20.0 » $Na_2HPO_4$. | 9.5 | 18.6 | 0.51 |
| 40.0 » $KH_2PO_4$ . | 0.3 | 17.8 | 0.02 |

Our experiments give the following results:

The glucose yields increasing amounts of acids as we go from the most acidic medium (5 m.e. HCl) to the most alkaline (40 m.e. NaOH) whereas the yield in carbon dioxide reaches a maximum in the presence of calcium carbonate, that is, at a reaction which must have been about neutral. The carbon dioxide is also high where no electrolyte was added although the reaction was here neutral only at the beginning.

The significant thing in the table is the fact that the acids/$CO_2$ ratios increase from the most acid to the most alkaline side. This is in agreement with our experience with humus and supports our explanation of the fact that a high acidoid content goes with a high base status.

The effect of the phosphates on the oxidation is very striking especially in the case of the primary potassium salt which causes an almost complete oxidation to carbon dioxide.

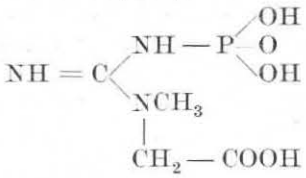
It should be added that large quantities of acids were found to be formed in an alkaline sugar solution without peroxide. In such cases the solution turns brown (»artificial humus») as if the energy

of the activated sugar molecules, in the absence of an oxidant, spends itself through a polymerization.

In another experiment we used sodium lactate instead of glucose and found 32.4 m.e. $CO_2$ with peroxide alone, 1.0 m.e. in the presence of 5 m.mols NaOH, 29.3 m.e. in the presence of 10 m.mols $Na_2HPO_4$, and 36.8 m.e. $CO_2$ in the presence of 10 m.mols $KH_2PO_4$. This shows that lactic acid is quite stable toward peroxide in an alkaline medium. Whether this is entirely due to the fact that the peroxide itself is unstable in alkaline solution must be left undecided. The fact remains that an alkaline medium favors the formation and accumulation of acids, in sugar as in plant materials and humus.

In our opinion the acid-base condition plays a greater role as a regulator of oxidation processes than is generally recognized. It seems very probable that fluctuations between alkalinity and acidity constitute the prime regulator of muscle respiration. Since the discovery of phosphocreatine (phosphagen) and its role in vital processes by the EGGLETONS (1927) our concepts of muscle physiology have, in the words of HILL (1932), been revolutionized. When phosphocreatine breaks down in the stimulated muscle forming creatine and phosphoric acid it yields, in addition to energy (120 calories per gram phosphoric acid), an alkaline reaction. This increase in alkalinity is, in our opinion, of fundamental importance for the respiratory processes which follow. But let us first see where the alkalinity comes from.

The acid residue of conjugated phosphoric acid ($R \cdot H_2PO_4$ and $R : HPO_4$) is much stronger than in the corresponding free ions ($H_2PO_4$ and $HPO_4^{--}$ because in the former there is no extra negative charge to suppress the dissociation of the H ions. We meet with the same effect in our amphoteric phosphates which have a low isoelectric point and which possess a high capacity to bind base at pH 7 all due to an increase in the dissociation of the acid residue. When such phosphates are hydrolysed we get an extra increment of base due to the fact that the phosphoric acid changes from a strong to a weak acid. The phosphoric acid in phosphocreatine



is porbably completely »neutralized» at the pH prevailing in the muscle and will, therefore, exist in the form of the ion R—NH—$PO_3^{--}$. After the hydrolysis the phosphoric acid will occur as a mixture of the two ions $H_2PO_4^-$ and $HPO_4^{--}$. We get, therefore, an extra increment of base by a reaction which we might formulate as follow:

$$R - NH - PO_3K_2 + 2\,H_2O \rightarrow$$
$$R - NH_2 + KH_2PO_4 + KOH.$$

The liberated base may then be assumed to combine with the enolized glucose which probably always exists in small amounts. This disturbs the equilibrium between the enolized and the normal form of glucose resulting in the formation of more of the enol form. If we assume the salt of the 3—4 dienol to be formed we can account for the appearance of lactic acid by an intramolecular oxidation-reduction thus:

$$\begin{matrix} CH_2OH - CHOH - COK \\ \\ CH_2OH - CHOH - COK \end{matrix} \quad \rightarrow \quad -2\,CH_3 - CHOH - COOK$$

By this mechanism the engine can be started promptly without having to wait to be fired up by oxygen. But the mechanism is only a primer which rapidly runs down and which must be wound up again if it is to work continuously. This winding up consists in the resynthesis of phosphocreatin at the expence of the energy formed by the lactic acid formation. But the formation of lactic acid and the resynthesis of phosphocreatine must increase the acidity. This might be assumed to release the mechanism whereby the ventilation and circulation are increased and the lactic acid is oxidized (the organism gets its »second wind»). In this complete oxidation the phosphate ions may be suspected to have a catalytic effect.

We can thus picture the respiratory mechanism to be controlled by oscillations between alkalinity and acidity, the magniude of these oscillations being governed by the intensities of the impulses which cause the exothermic breakdown of phosphocreatine. Each quantum of alkalinity produced must activate and cause a definite quantity of glucose to be transformed into lactic acid.

## Oxidation and ammonia fixation.

The previously found relationships (cf. part IV of this series) between the humus and litter acidoids and the nondialyseable (and nonexchangeable) nitrogen might be summed up as follows:

1. The nitrogen content of humus and, with some exception, of litter increases with an increase in the acidoid content.
2. When humus is treated with ammonia a certain amount of the latter becomes fixed in a nondialyseable form, the amount thus fixed being proportional to the acidoid content of the humus.
3. The fixation of ammonia reduces the strength of the acidoids, i.e., raises the $pH_a$ and the isoelectric point, just as an abnormally

high nitrogen content in the natural litter is associated with a weak acidoid.

These observations give rise to many questions such as the following:

1. What are the conditions which favor the fixation of ammonia and what is the maximum amount fixable?
2. Which are the ammonia fixing groups and in what form does the ammonia enter the organic complex (amide, amino, heterocyclic or otherwise)?
3. How does the ammonia modify the physical, chemical and biochemical properties of the litter and humus complex?

The present paper deals with the first of these questions. The work was undertaken primarily with the object of finding a suitable method for preparing ammoniated humus on a large scale for investigations dealing with the second and third questions. The aim was to fix as much ammonia as possible, and having found 1. that a high fixation goes with a high acidoid content and 2. that oxidation in alkaline solution leads to a high acidoid content we decided to study the effect of mild oxidizing agents on the fixation of ammonia.

The ammonia fixation was studied on the $F_2$ sample (same as in table 29). The investigation was carried out in two series, one, the E-series, at room temperature and the other, the F-series, under pressure at 132° C.

*E-series*: 20 grams humus (oven dry basis) were placed in six suction flasks of 2 liters capacity together with 400 cc concentrated ammonia. Flask 1 received no peroxide whereas flasks 2, 3, 4, 5 and 6 received 5, 10, 20, 30 and 40 cc portions respectively of the 30 % solution. The peroxide was introduced in 5 cc portions at intervals of 24 hours as previously described.

Flask n:o 6, which received eight 5 cc portions of peroxide, was connected through the side tube to a manometer and the pressure was recorded. After each addition of peroxide the pressure increased rapidly, attaining a maximum after one to two hours, after which it fell considerably during the night, thus showing that the liberated oxygen was consumed by the humus. At the conclusion of the experiment on the ninth day the pressure measured 480 mm. of mercury.

The suspensions were now evaporated to dryness in a current of warm air at a temperature about 40° C. The residues were then electrodialysed until free from all diffusible acids and bases and again dried at 40°. The samples thus obtained were then studied with respect to the properties given in table 39, series E.

F-series: 20 grams of the humus and 400 cc concentrated ammonia were heated in a steel bomb at 132° C. for 18 hours, 1. without hydrogen peroxide, 2. with 10 cc and 3. with 20 cc 30 % peroxide solution. The samples were then dried and electrodialysed as explained. The results are shown in table 39, series F.

For comparison the corresponding data for the untreated, electrodialysed $F_2$ sample is also given (table 39, O). The titration curves are shown in fig. 38.

The table gives the following data:

*The nitrogen content.* In the absence of peroxide the nondisplaceable nitrogen has increased from 1.81 to 3.53 percent in the cold series and to 5.34 percent in the heated series. In the oxidized systems the ammonia fixation increases with the increase in peroxide. In the cold series the increase due to the peroxide mounts to nearly 2 percent (system E 6) and in the heated series to over 1 percent (system F 3).

Table 39. *Nitrogen and acidoid content and $pH_u$ of electrodialysed podzol humus ($F_2$) after treatment with ammonia and hydrogen peroxide (20 grams humus + 400 cc concentrated ammonia)*:

E. At room temperature for 8 days.
F. In steel bomb at 132° C for 18 hours.
O. Untreated.

| No | $H_2O_2$ 30 % sol. | Org. matter in residue | Per 100 g. organic matter: Anode acids | $A$(pH 7) | $A$(pH 10) | $\frac{A(pH\ 10)}{A(pH\ 7)}$ | N | $pH_u$ in N. KCl |
|---|---|---|---|---|---|---|---|---|
| | cc | g. | m.e. | m.e. | m.e. | | % | |
| E 1 | 0 | 16.3 | 48 | 111 | 219 | 1.97 | 3.53 | 2.85 |
| E 2 | 5 | 16.4 | 86 | 117 | 214 | 1.83 | 4.37 | 2.84 |
| E 3 | 10 | 16.1 | 105 | 119 | 211 | 1.77 | 4.85 | 2.75 |
| E 4 | 20 | 14.8 | 135 | 112 | 203 | 1.81 | 5.30 | 2.73 |
| E 5 | 30 | 13.3 | 172 | 112 | 189 | 1.69 | 5.32 | 2.69 |
| E 6 | 40 | 12.7 | 218 | 116 | 188 | 1.62 | 5.51 | 2.67 |
| F 1 | 0 | 14.2 | 95 | 94 | 241 | 2.56 | 5.34 | 3.78 |
| F 2 | 10 | 13.3 | 127 | 103 | 233 | 2.26 | 6.22 | 3.56 |
| F 3 | 20 | 12.0 | 196 | 111 | 235 | 2.12 | 6.37 | 3.45 |
| O | — | 18.5 | 16 | 127 | 276 | 2.17 | 1.81 | 2.08 |

Fuestel and Byers (1933) found the nonammoniacal nitrogen to increase by 3.05 percent when they heated a sawgrass peat in 19.6 percent ammonia solution to a temperature of 150° C for 3 hours. Ehrenberg and Heimann (1930) who studied the fixation of ammonia in the presence of oxygen and various catalyzers at still higher temperature and pressure found the *total* nitrogen to reach 15 percent. By heating a Na-humate of the Mölner humus with concentrated ammonia to 132° C. for 18 hours we obtained a product containing 8.52 percent of *nondisplaceable* nitrogen. This and other specially prepared humates are now being studied and will be discussed in a forthcoming paper. Other experiments have shown

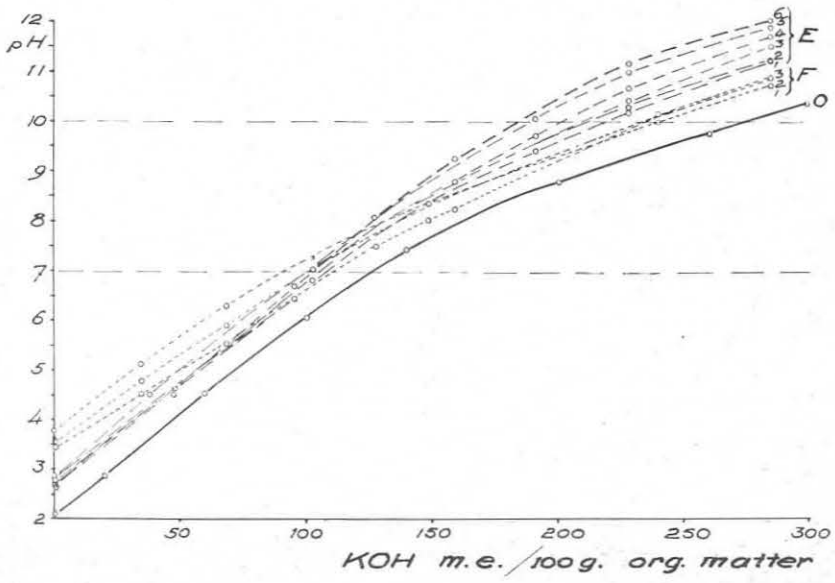



Fig. 38. The titration curves of the ammoniated samples in table 39.

that humus will fix measurable amounts of ammonia in the cold even from very dilute solutions.

*The acids.* These represent the titration values of the anode solution. The quantities of these acids are of the same order of magnitude (in most cases somewhat lower) as the quantities of *a*-acids in the corresponding systems in the A and C series in table 29. The anode solutions were more or less colored and contained, therefore, some $\beta$-acids. Ammonia being a much weaker base than NaOH, we would naturally expect smaller amounts of *a* and $\beta$-acids to be formed in the ammoniacal systems.

*The acidoids.* The remarkable thing about the acidoid content per 100 grams ammoniated humus is that this has decreased in every system. This is in agreement with our earlier findings that the fixed ammonia masks the acidoid activity, probably by an intramolecular »neutralization» (zwitter ion formation) as in the proteins. At pH 7 the acidoid content is about equally suppressed in series E. In series F this suppression is greatest without peroxide and is smallest with a maximum of peroxide. We have here obviously to do with two opposing tendencies: the oxidation increases the acidoid content and the ammonia fixation masks the acidoid activity.

At pH 10 the acidoid content decreases, here as in table 30, with an increase in peroxide. This trend is evidently, as already ex-

plained, due to a progressive destruction of the weakly acidic, phenolic groups. This expresses itself more clearly in the ratio A(pH 10)/A(pH 7) which decreases in both series with an increase in peroxide.

This ratio is wider in the ammoniated systems than in the corresponding systems in table 30, two of the heated systems showing a wider ratio than the untreated sample. This is very significant for it indicates that the fixed ammonia masks the stronger carboxyl groups more than it masks the phenolic groups. This is also what one would expect because if the fixed ammonia has as weak basic properties as free ammonia it will not combine with the weakest acidoid groups and, furthermore, the intramolecular compound between the strong acidoid groups and the fixed ammonia groups must hydrolyse at a high pH. The compound will, therefore, buffer at a higher pH than the free acidoid. The titration curve of such a compound (zwitter ion) will be an expression of the hydrolysis constant of the compound. In the terminology of BRÖNSTED we are titrating the acid $RNH_3^+$ as expressed by the equation:

$$CO_2^- \cdot R \cdot NH_3^+ + K^+ + OH^- = CO_2^- \cdot R \cdot NH_2 + K^+ + H_2O.$$

If this explanation is correct it might be possible to prove it by the FOREMAN reaction (1920). FOREMAN found that if amino acids and even ammonium chloride be titrated with alcoholic KOH in 85 percent alcohol the result will be the same as when a corresponding amount of free acid is titrated: the amino group and ammonia itself is not a base in the alcoholic solution.

We decided to try this reaction but we could obviously not use indicators in so darkly colored solutions and the electrometric method is not applicable to strongly alcoholic solutions. We tried, therefore, the following method which seemed to yield at least approximately reliable results. Half a gram samples of $F_2$ (table 39) and of C 2 (table 29) (the only two samples available in sufficient quantities) were placed in 10 cc water plus aqueous KOH in one series of tubes and in 10 cc 85 percent alcohol plus alcoholic KOH in another series. The tubes were shaken in the machine for 2 hours and left to stand over night for the sediment to settle. Two cc were then withdrawn and diluted to 10 cc, with water in the case of the alcoholic series, and with water and sufficient alcohol to make the water series contain 17 percent alcohol. The 2 cc from the reaction tubes did, of course, contain some humate but the alcoholic extract was not very dark colored.

The pH in each of the four diluted series was then determined by the quinhydrone method (the glass electrode used in all other determinations in this work was being repaired at this time). The titration curves are shown in fig. 39. We note that the capacity to bind base is considerably greater in the alcoholic series and that the

difference becomes smaller at high pH where the masking effect of the ammonia should, theoretically, also be less. The carrying over of some humate in the diluted series must, of course, also cause the curves to converge at high pH where the humus is more soluble. If no humus had been removed by sedimentation the diluted systems would have been alike and the curves identical.

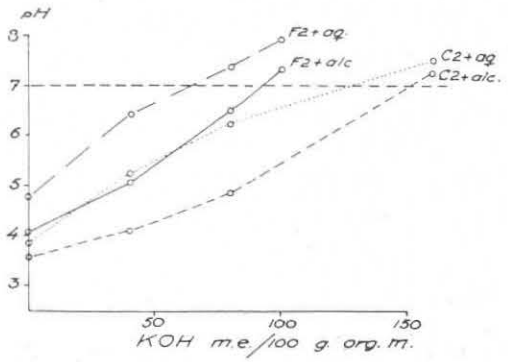



Fig. 39. The capacity of two of the samples to bind base in water and in 85 % alcohol.

An interpolation of the curves at pH 7 shows that the C 2 sample (1.67 % N) binds about 20 percent more base in 85 percent alcohol than in water, whereas the ammoniated (and heated) F 2 sample (6.22 % N) binds about 46 percent more base in the alcoholic solution. Note that the $pH_u$ is much lower in the alcoholic solution than in water especially in the case of the F 2 sample. It should also be noted that these solutions contained no KCl and that the final dilution was 1 : 100 instead of the usual 1 : 10.

*The $pH_u$.* This is very much higher in the ammoniated samples, especially in the heated series, than in the original humus (cf. fig. 40). This is in agreement with previous observations (cf. Part IV) that an »abnormally» high nitrogen content in the original litter and humus as well as in the ammoniated gives rise to a high $pH_u$, an indication that the nitrogen enters the complex as a basic group thus raising its isoelectric point. The fact that the $pH_u$ in table 39 decreases with an increase in nitrogen might seem to contradict this explanation, but it must be assumed that the oxidation has increased both the strength and the true concentration of the acidoids although the apparent acidoid content at pH 7 has been decreased by the masking effect of the ammonia.

## Ammonia fixation by the unoxidized and auto-oxidized samples.

Two grams of each of the electrodialysed samples described in tables 32 to 36 were placed in porcelain crucibles, covered with concentrated ammonia and placed in a desiccator over concentrated ammonia. The desiccator was evacuated until the ammonia began to boil. After standing for one week the ammonia was evaporated and the samples dried at 60° C. The free ammonia was then determined by distillation with magnesium oxide and the total nitrogen was found by the KJELLDAL method. The difference between the nonammonia nitrogen and the original nitrogen content gave the amount of ammonia fixed by the treatment. Table 40 gives the results.

The results are startling but very instructive. On the basis of our previous work we had anticipated a greater fixation in the oxidized samples but the results were the opposite: a greater fixation in the unoxidized samples, in the »lignin» as well as the whole samples. Another startling result is the fact that the genetically highly oxidized Mölner fixes much less ammonia (0.94 %) than the podzol series of samples which fix from 1.73 to 2.02 % in the unoxidized condition.

We have previously suggested (Part IV) that the ammonia fixation might be proportional to the acidoid content, but this is evidently not the case — as it cannot be if a high degree of oxidation suppresses the power to fix ammonia. In our previous work there was evidence of such a proportionallity in the case of the Häggbygget series of samples. But by the method now used (less aeration, lower temperature) these samples cannot be said to show any such proportionallity. The proportionallity between the acidoid content and the original content of nitrogen is, however, fairly close.

We note also that the fixation of ammonia by the unoxidized »lignin» samples is not appreciably greater than that by the whole samples although the acidoid content of the former is much greater. The same is true of straw and straw lignin.

The oxidized samples fix, as already noted, much less ammonia than the unoxidized. The difference seems to be greatest in the youngest plant materials, e.g., in the V sample and in the straw and straw lignin. The difference is (cellulose not counted) smallest in the Mölner sample.

We have here an important clue to the mechanism of the ammonia fixation. What conclusions does this clue lead us to?

If we recall that oxidation in the *presence* of ammonia leads to increased ammonia fixation we can sum up the facts as follows:

*Simultaneous oxidation favors, and accomplished oxidation suppresses the fixation of ammonia.*

Table 40. *The ammonia fixation of the unoxidized and auto-oxidized samples in tables 32 to 36.*

| Sample | Acidoids in original samples | | N in original samples | | N in ammoniated samples | | Difference = $NH_3$-fixation | |
|---|---|---|---|---|---|---|---|---|
| | unoxidized | oxidized | unoxidized a | oxidized b | unoxidized c | oxidized d | unoxidized c–a | oxidized d–b |
| | m.e. | m.e. | % | % | % | % | % | % |
| A: Whole samples. | | | | | | | | |
| V Häggbygget | 62 | 75 | 1.05 | 0.67 | 2.79 | 0.95 | 1.74 | 0.28 |
| $F_1$ » | 87 | 111 | 1.24 | 0.96 | 2.97 | 1.52 | 1.73 | 0.56 |
| $F_2$ » | 127 | 156 | 1.81 | 1.64 | 3.83 | 2.32 | 2.02 | 0.68 |
| H » | 157 | 185 | 2.03 | 1.96 | 3.99 | 2.71 | 1.96 | 0.75 |
| Mölner | 319 | 297 | 3.94 | 4.05 | 4.88 | 4.76 | 0.94 | 0.71 |
| Annerstad | 121 | 167 | 1.20 | 0.97 | 2.21 | 1.62 | 1.01 | 0.65 |
| B: »Lignin» samples. | | | | | | | | |
| V Häggbygget | 111 | 249 | 1.79 | 1.98 | 3.72 | 3.13 | 1.93 | 1.15 |
| $F_1$ » | 177 | 295 | 2.03 | 2.34 | 3.83 | 3.43 | 1.80 | 1.09 |
| H » | 234 | 317 | 2.29 | 2.46 | 4.29 | 3.56 | 2.00 | 1.10 |
| Mölner | 368 | 357 | 3.82 | 3.74 | 4.98 | 4.14 | 1.16 | 0.40 |
| C: Wheat straw, lignin, casein-lignate and cellulose. | | | | | | | | |
| Straw | 21 | 35 | 0.61 | 0.51 | 1.33 | 0.54 | 0.72 | 0.03 |
| » lignin | 74 | 201 | 2.14 | 2.62 | 2.97 | 2.69 | 0.83 | 0.07 |
| Casein-lignate | — | 190 | — | 4.79 | — | 5.33 | — | 0.54 |
| Cellulose | 16 | 18 | 0.03 | 0.01 | 0.11 | 0.04 | 0.08 | 0.03 |

The most obvious conclusion of this is that the group in the molecule which binds the ammonia is an intermediate oxidation group, possibly a carbonyl group. The fully oxidized carboxyl group does not fix the ammonia. The ammonia fixing group is fairly stable because it exists in the natural materials. The bond between this group and ammonia is very stable because the ammonia, once it has been fixed, cannot be »oxidized out». (Note the increase of nitrogen in the oxidized »lignins». The decrease in nitrogen in the oxidized whole samples is, as already pointed out, due to a solvation and loss of the ligno-nitrogenous complex. Recall also that ammonia stays fixed even against the action of peroxide.) The fixed ammonia has basic properties because it raises the $pH_u$ (and the I.E.P.) and lowers the base binding capacity; it must, therefore, form an inner salt with the acidoid groups. The ammoniated complex is, probably for this reason, more stable, i.e., less highly solvated, and is more readily precipitated by alkali chlorides and by alcohol (data to be published later).

On the basis of the evidence here presented we consider the assumption justified that the ligno-nitrogenous complex is formed by an interaction between lignin and ammonia and possibly other decomposition products of the proteins, especially the aromatic amines. Lignin would then function as a conserver of nitrogen by tying up its soluble compounds in a more stable form much as the acidoids serve to conserve the soluble bases. Since an already oxidized humus fixes less ammonia we should expect a high nitrogen content to go with a high acidoid content only when ample amounts of ammonia were formed *during* the oxidation of the humus.

## Oxidation with nitric acid.

Twenty grams of the Häggbygget and Mölner samples were treated for 18 hours at room temperature with 100 cc concentrated nitric acid diluted to 200 cc with water. The mixtures which got warm were cooled in water while gases were escaping. The samples were then thoroughly washed, dried and electrodialysed. The results of the analysis are given in table 41.

Table 41. *The acidoid and nitrogen content and the $pH_u$ of the samples after the nitric acid (1 : 1) treatment.*

| Sample | Per 100 g. organic matter | | | | $pH_u$ in N. KCl |
|---|---|---|---|---|---|
| | *A* (pH 7) | *A* (pH 10) | $\frac{A\ (pH\ 10)}{A\ (pH\ 7)}$ | N % | |
| Häggbygget V ..... | 111 | 208 | 1.88 | 1.91 | 2.07 |
| » $F_1$ ..... | 157 | 268 | 1.71 | 2.62 | 1.96 |
| » $F_2$ ..... | 197 | 345 | 1.75 | 3.28 | 1.88 |
| » H ..... | 204 | 360 | 1.77 | 3.22 | 1.88 |
| Mölner ..... | 293 | 437 | 1.49 | 5.01 | 1.69 |

The results are of interest in that, as far as the podzol series of samples are concerned, we have here a case where the acidoid content has been increased and the $pH_u$ has been lowered at the same time the nitrogen content has been increased (comp. table 32). The increase in nitrogen does not here disturb the »regular» relationship between the acidoid content and the $pH_u$ because the latter values fit the curve in fig. 40 very closely. The nitrogen has probably here entered the complex in the form of nitroso compounds as explained in part IV. The nitric acid was partly reduced to nitrous acid and this has then combined with secondary amino bases to form nitroso compounds. The added nitrogen is, therefore, here not basic and does not raise the $pH_u$. The primary amino groups must have been deaminized by the nitrous acid, as indicated by the evolution

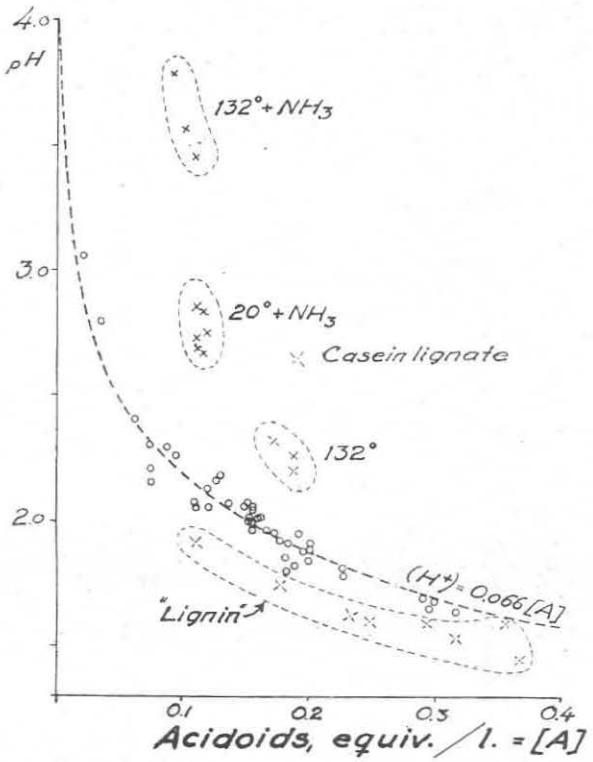



Fig. 40. The relationship between the $pH_u$ and the acidoid content of all samples.

of gas, so that the treated samples must be poorer in basic groups than the original.

The ratios $A$(pH 10)/$A$(pH 7) are here higher than in table 33 b. This might possibly be due to an increase in the acid strength of the phenolic groups due to the entrance of electronegative N-groups in the same way that picric acid is a stronger acid than phenol.

The Mölner humus which is already highly oxidized shows no increase in its capacity to bind base.

## The $pH_u$.

Fig. 40 gives the pH in N. 1 KCl of the electrodialysed samples (the »ultimate» pH or $pH_u$) as a function of the acidoid concentration expressed in equivalents per liter (in the 1:10 suspensions). The acidoids were determined by titration to pH 7 in a N. 1 KCl solution and not in water as in our previous work (Part IV). Since the colloids bind considerably more base in a salt solution than in water the

values for the acidoid concentration [A] become correspondingly higher. The value of the constant c in the linear equation

$$(H^+) = c[A]$$

should, therefore, this time be smaller than our previously found constant of 0.067. In the present case we find however a but slightly lower average value for c:

$$(H^+)/[A] = 0.066$$

This includes only the values represented by circles in the figure. That this new c value is nearly as large as the previous value might be due 1. to the fact that we this time employed the glass electrode whereas the former pH determinations were made by the quinhydrone method, and 2. that most of the samples were this time oxidized to a high acidoid content without a corresponding increase in (basic) nitrogen.

We have not been able to explain why this equation should apply to humus in general. It seems as if humus assumes a stable form in which a definite proportion of the strongest acidoid groups are compensated by basic nitrogen. We can however easely understand why an abnormally high content of basic nitrogen must cause the $pH_u$ to be out of proportion high with respect to the acidoid content. It requires relatively little base to raise the pH by one unit, from pH 2 to 3 for example. By doing this we reduce the H ion concentration to one tenth the original while the free acidoid concentration suffers a much smaller reduction. Hence it is obvious that an abnormally high content of basic nitrogen must lead to an abnormally high $pH_u$ with respect to the acidoid content. Note the position of the ammoniated samples in fig. 40.

The position of the A series, in the figure (132°, cf. table 29) indicates that the heating with alkali causes a destruction of the strongest acidoid groups. The low position of the »lignin» samples has already been discussed. The position of the »casein lignate» in the figure is another evidence against the ligno-protein theory of humus by WAKSMAN.

## Applications to soil conditions.

The most important factor in the weathering of soil minerals is the base status for it is this which determines the nature of the hydrolysis and of the quality of the solvation and eluviation, and which therefore, determines the composition and properties of the residual weathered complex. By way of analogy the same can be said of the »weathering» of soil organic matter. The base status determines the composition and properties of the humified complex. A

high capacity to bind base and a high nitrogen content go with a high base status and vice versa (cf. Part III).

The nitrogen in humus has been found to increase with increasing pH (HESSELMAN). In a climatic series of soils WAKSMAN reports twice as much »protein» in a chernozem humus and three times as much in a serozem humus as compared to a podzol humus (1938, table 19). The nitrogen content of humus has also been found to increase with the depth of the podzol profile. This is evidently the result of an anionic solvation and eluviation of the ligno-nitrogenous acidoids and $\beta$-acids which then become isoelectrically precipitated in combination with sesquioxides in the B-horizon. It is obviously the most highly oxidized fractions possessing the highest acidoid content and therefore also the highest nitrogen content which are thus eluviated. This explains also why the base exchange capacities of the fractions extracted as alkali humate increase with the depth of the podzol profile (BORATYŃSKI and MATTSON).

## Activated humus and gleization.

The blue-gray soil horizon often developed under standing water and known as glei formation seems to be associated with a sour humus layer (peat or podzol) and a high base status in the glei horizon. From a purely chemical point of view the following deductions might be made.

When sour humus is carried into the mineral soil it becomes more or less saturated with bases and is at the same time activated to a higher reduction potential. In the absence of oxygen it reduces the ferric iron in the soil which thereby assumes the characteristic blue-gray color of the glei horizon.

ALBRECHT (1941) who has studied the problem from a microbiological point of view emphasizes the importance of a high Ca saturation in the glei horizon which he considers essential for anaerobiosis. ALBRECHT, whose suggestions are very interesting, states: »It (the Ca saturation) raises the question whether the profile horizons above that of glei formation are not simply of too low a degree of calcium saturation for microbial activity, so that the percolating organic matter is not of service until it has moved downward to the horizon of sufficient calcium saturation and the corresponding relative saturation of other bases.» It would be interesting to know if humus which has been formed under the conditions of a high base status would stimulate the anaerobiosis in the Ca saturated horizon to the same extent to which it was found to be stimulated by the genetically sour humus. Do anaerobic bacteria at a high pH utilize genetically base saturated humus as readily as genetically unsaturated humus as a source of energy? From the chemical behavior of humus we should not think so.

## Heavy liming and toxicity.

Heavily limed acid muck and peat soils have been found to be toxic to plants. It occurred to us that this toxicity might be due to the alkaline activation of the reducing power of the humus. Experiments with barley plants proved the anaerobically activated (treatment with 2 N. NaOH) Häggbygget H sample to be toxic when one percent was added to the nutrient solution in the form of neutral Na-humate whereas the activated and oxidized Mölner humate showed no toxic effect. The toxic humate stopped the growth of the roots, and the dry weight, after three weeks, was only slightly more than 50 percent of the weight of the normal plants. This problem deserves a systematic study.

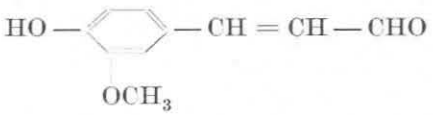
## Theoretical discussion.

The capacity to bind base, to fix ammonia and to undergo auto-oxidation must be looked upon as the most important chemical properties of soil organic matter. We have found these properties to be intimately related to one another and we have found them to be the properties of the lignin or ligno-nitrogenous fraction of the organic complex. A knowledge of the mechanism of these properties must, therefore, hinge on our knowledge of the constitution of lignin. Despite much fruitful work we still know less about lignin than about the other major constituents of plants, the carbohydrates and the proteins. The reason for this is that the lignins do not hydrolize into simple, well defined derivatives.

Most authors are agreed upon the aromatic nature of the main structural units in the lignin molecule. The presence of phenol hydroxyls, some of which are methylated, is well established.

According to FUCHS (1931) heterocyclic oxygen is also present and this can be replaced by ammonia.

The assumption of KLASON that coniferyl aldehyde



constitutes the main structural unit of lignin is, according to HÄGGLUND, supported by many of its chemical properties. Thus the ethylene groups combine with sulphites to form lignosulphonic acids and the aldehyde carbonyl combines with aromatic amines giving the characteristic color reactions of lignin. Lignosulphonic acid reacts with $\beta$-naphthylamine to form an anilic acid which, according to KLASON, exists in two modifications:

$$R \cdot CH_2 \cdot CH\begin{matrix}\diagup SO_2O \diagdown \\ \diagdown CH{:}NH \cdot C_{10}H_7 \diagup\end{matrix} \qquad \text{I}$$

and

$$R \cdot CH_2 \cdot CH\begin{matrix}\diagup SO_2OH \\ \diagdown CH{:}N \cdot C_{10}H_7\end{matrix} \qquad \text{II}$$

The first of these is insoluble and stable in acid solutions. The second compound, which is soluble and which is formed from the first by treatment with alkali, combines, according to HÄGGLUND, in neutral solutions with a second molecule of naphthylamin. This second molecule is however easely displaced both by acid and alkali and is assumed to form a normal salt with the lignosulphonic acid.

We have here a case which is analogous to the power of humus acidoids 1. to fix ammonia in a nondisplaceable form and 2. to bind displaceable ammonium ions. The fixed ammonia not only lowers the capacity of the acidoid to bind base (as must be the case with naphthylamin in compound I) but it also suppresses the solubility of the alkali humates which, in the ammoniated condition, are precipitated by concentrated alkali chloride solutions and by relatively less concentrated alcohol.

The presence of unsaturated aldehyde side chains would account for the alkaline auto-oxidation and for the resulting formation of carboxyl acids. In this connection it is of interest to recall that the oxidative rupture of condenced phenols leads to the formation of similar side chains. Thus the careful oxidation of $\alpha$-naphthol yields o-cinnamo-carboxylic acid:

$$C_6H_4\begin{matrix}\diagup COOH \\ \diagdown CH = CH - COOH\end{matrix}$$

and $\beta$-naphthol yields o-carboxyl-phenyl-glyoxylic acid:

$$C_6H_4\begin{matrix}\diagup COOH \\ \diagdown CO - COOH\end{matrix}$$

Fig. 41 shows the titration curves of $\beta$-naphthol before and after a partial oxidation with $KMnO_4$ in cold, alkaline solution.

We have seen that the oxidation of lignin and humus leads to a decrease of the weak acidoid groups, assumedly phenol hydroxyl, and to an increase of the stronger groups, assumedly carboxyl. Now if the lignin molecule is made up of a network of condenced, hydroxylated benzene rings, as pictured by FUCHS and others, the following question suggests itself:

Is the oxidative transformation of lignin of the same nature as the aforementioned transformation of the naphthols? If so it would account for a number of things such as the disappearance of the phe-

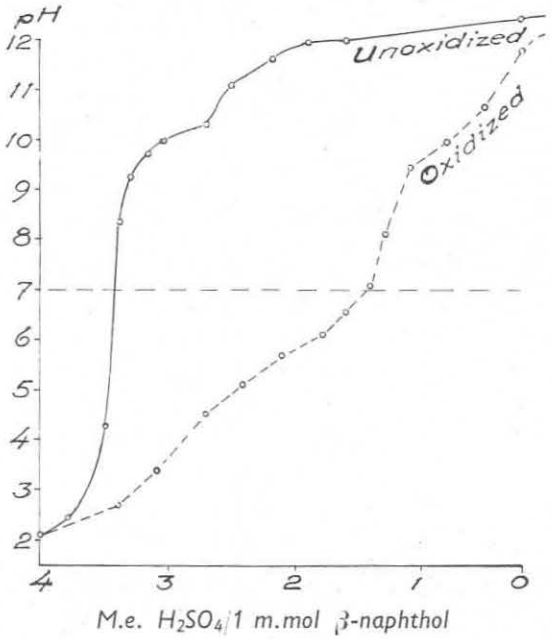



Fig. 41. The titration of one millimol of $\beta$-naphthol before and after a partial oxidation by $KMnO_4$ in alkaline solution. Oxidation mixture: $C_{10}H_7OH + 2KOH - 4KMnO_4$.

nol hydroxyls, the increase in the content of strong acidoids, the splitting off of the acid-soluble $\beta$-acids, the formation of simple acids such as the polycarboxyl benzene acids, acetic and formic acids (from the side chains) and carbon dioxide. But where does then the fixation of ammonia come in? Do the unsaturated and carbonyl carrying side chains bind the ammonia in some way? In support of this possibility we might point to the fact that highly auto-oxidized humus and »lignin» has lost much of its power to fix ammonia.

It is conceivable that the disappearance upon oxidation of the weakly acidic phenol hydroxyls is not due to their destruction but to a strengthening through the formation of oxy-quinones as in ELLERS (1925) condenced phenols. The hydroxyl groups of the oxy-quinonens are often as strongly acidic as the carboxyl acids. But this type of oxidation would not account for the various soluble products nor would it account for the fixation of ammonia unless we assume the quinonic oxygen to be substituted for ammonia as it is, in certain cases, by aniline (with the aid of a simultaneous oxidation process). ELLER assigns, however, no place for nitrogen in his formula.

We are now studying the auto-oxidation, titration and ammonia fixation of humus and lignin in combination with various radicles such as phenols, aromatic amines, sulphonic acid, thioglycollic acid and other »lignin reagents». The results of this work will be published in this series of papers.

Concerning the mechanism of the formation of humus acidoids it cannot be said that it has yet been proven that these are products of microbiological activity. In proportion to the lignin content the original litter seems to possess about as high an acidoid content as that of a sour humus. Only in humus having a high base status is the increase in acidoid content considerably greater than the relative increase in the lignin content due to the decomposition. This increase in acidoids must be credited to an oxidation of the lignin. How far this oxidation is due to an activation and auto-oxidation of the lignin at the higher base status and how far it is due to microbial oxidases is not known.

Cellulose does not seem to form acidoids and the proteins bind only small amounts of base even at pH as high as 8 or 9. Both form soluble products of hydrolysis, which are easely decomposed. Materials from young plants, which are high in protein, possess, like the »protein lignates» and »humates», a high $pH_u$, i.e., possess a high I.E.P. Only lignin or the ligno-nitrogenous complex seems to develope strong acidoid properties.

Authors disagree as to nature of the materials which condense to form lignin even more than they disagree as to the form of the condensation, whether aliphatic, heterocyclic or carbocyclic. We believe that the idea advanced by FUCHS, that the pectins are the precursors of lignin, has much in its favor.

Young wheat and barley plants develope a fairly high acidoid content and a still higher content of dialyseable organic acids. Later, when the plants mature and the lignification is completed, there is a decrease in both acids and acidoids, which is accompanied by a corresponding decrease in bases.[1] Now the young wheat and barley plants need active materials, soluble and colloidal, in order to maintain the necessary osmotic and potential differences. After the rapid growth the activities subside, but now the plants need rigidity and strength. The inference is that the plants utilize the active materials to construct the, chemically more indifferent, structural material, the lignin. If acids, pectic and others, participate in the condensation to lignin a certain number of acidic groups may be assumed to remain intact. This number may be assumed to be determined by the base status of the plant just as the acidoid content of humus is determined by the base status of the soil. The nature of the lignin, which varies in different species of plants,

[1] From data to be published in this series.

would then, like the nature of humus, be intimately tied up with the acid-base condition.

## Summary.

The partial oxidation of soil organic matter by hydrogen peroxide and by alkaline auto-oxidation in an atmosphere of oxygen leads to the formation of

1. a higher acidoid content of the residue;
2. dark colored »$\beta$-acids» which are soluble in acids but precipitated by salts of aluminium or iron and which possess a very great capacity to bind base;
3. simple organic acids and
4. carbon dioxide.

The ratio of carbon dioxide to the increase in total acidity is greatest in an acid medium.

Alkali activates the humus and increases the acid and acidoid content without the addition of oxygen. The activated material absorbs oxygen greedily, at first very rapidly then more gradually. The ratio $CO_2$ $O_2$ is much lower during the rapid than during the slow oxidation.

The acidic properties have been found to belong to the lignin fraction. Proteinated lignin (»humus nucleus») binds less base than free lignin if both are equally oxidized.

Fresh and slightly humified litter and unoxidized lignin possess a very high content of weak acidoids (assumedly due to phenol hydroxyls) which buffer strongly above a pH of about 9. These acidoids are destroyed (or strengthened) by the oxidation which yields relatively strong acids and acidoids.

The fixation of ammonia is increased by a *simultaneous* oxidation whereas as an *accomplished* oxidation, whether in nature or in the laboratory, suppresses the fixation. The ammoniated samples bind less base than the untreated, but in the Foreman titration in alcohol there is a recovery in the capacity to bind base.

The $pH_u$ of the oxidized samples show a define relationship to the acidoid content, but the ammoniated and proteinated samples all yield abnormally high $pH_u$ values.

The results have been discussed in relation to soil conditions and in relation to the chemistry of lignin.

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